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JAN 7 1997 PAT. & TRADEMARK OFFICE

Attorney Docket **P0986P2**
PATENT

CERTIFICATION UNDER 37 CFR 1.10

EM 239 527 297 US: Express Mail Number 07 January 1997 **Date of Deposit**

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.

Sandra K.T. Sullivan
Sandra K. T. Sullivan

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOX PATENT APPLICATION
Assistant Commissioner of Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of

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Nancy Y. Chiang
Kyung Jin Kim
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Maria L. Rodrigues

For: **WSX RECEPTOR AGONIST ANTIBODIES**

1. **Type of Application**
This new application is for a(n) _ original X continuation-in-part application.

2. **Papers Enclosed Which Are Required For Filing Date Under 37 CFR 1.53(b)(Regular)**
155 pages of specification
3 pages of claims
1 page(s) of abstract
61 sheet(s) of drawings
[X] formal [ ] informal

3. **Declaration or Oath**
X The declaration of the inventor(s) [X] is enclosed [ ]signed [X]unsigned.

4. **Assignment**
X An Assignment of the invention to GENENTECH, INC. [] is enclosed with attached Recordation Form Cover Sheet [X] will follow.

Revised (10/13/96)

5. Fee Calculation (37 CFR 1.16)
The fee has been calculated as follows:

CLAIMS FOR FEE CALCULATION					
Number Filed		Number Extra		Rate	Basic Fee 37 CFR 1.16(a)
					$770.00
Total Claims	39	- 20 =	19	X $22.00	$418.00
Independent Claims	1	- 3 =	0	X $80.00	$0.00
__	Multiple dependent claim(s), if any			+ $260.00	$0.00
				Filing Fee Calculation	$1,188.00

6. Method of Payment of Fees
The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $1,188.00. **A duplicate copy of this transmittal is enclosed.**

7. Authorization to Charge Additional Fees
The Commissioner is hereby authorized to charge any additional fees required under 37 CFR §1.16 and 1.17, or credit overpayment to Deposit Account No. 07-0630. **A duplicate copy of this sheet is enclosed.**

8. Additional Papers Enclosed
[] Information Disclosure Statement (37 CFR §1.98) w/ PTO-1449 and citations
[X] Submission of "Sequence Listing", computer readable copy and/or amendment pertaining thereto for biological invention containing nucleotide and/or amino acid sequence and Sequence Statement.
[ ] Other:

Respectfully submitted,
GENENTECH, INC.

Date: 1/7/97

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WSX RECEPTOR AGONIST ANTIBODIES

CROSS REFERENCES

This application is a continuation-in-part of co-pending U.S. Application Serial No. 08/667,197 filed June 20, 1996, which is a continuation-in-part of co-pending U.S. Application Serial No. 08/585,005 filed January 11, 1996, which applications are incorporated herein by reference and to which applications priority is claimed under 35 USC §120.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention pertains generally to the WSX receptor. In particular, the invention relates to agonist antibodies which bind to and activate the WSX receptor.

Description of Related Art

A. HEMATOPOIESIS

The process of blood cell formation whereby red and white blood cells are replaced through the division of cells located in the bone marrow is called hematopoiesis. For a review of hematopoiesis see Dexter and Spooncer (*Ann. Rev. Cell Biol.* 3:423-441 (1987)).

There are many different types of blood cells which belong to distinct cell lineages. Along each lineage, there are cells at different stages of maturation. Mature blood cells are specialized for different functions. For example, erythrocytes are involved in O_2 and CO_2 transport; T and B lymphocytes are involved in cell and antibody mediated immune responses, respectively; platelets are required for blood clotting; and the granulocytes and macrophages act as general scavengers and accessory cells. Granulocytes can be further divided into basophils, eosinophils, neutrophils and mast cells.

Each of the various blood cell types arises from pluripotent or totipotent stem cells which are able to undergo self-renewal or give rise to progenitor cells or Colony Forming Units (CFU) that yield

a more limited array of cell types. As stem cells progressively lose their ability to self-renew, they become increasingly lineage restricted. It has been shown that stem cells can develop into multipotent cells (called "CFC-Mix" by Dexter and Spooncer, *supra*). Some of the CFC-Mix cells can undergo renewal whereas others lead to lineage-restricted progenitors which eventually develop into mature myeloid cells (*e.g.*, neutrophils, megakaryocytes, macrophages and basophils). Similarly, pluripotent stem cells are able to give rise to PreB and PreT lymphoid cell lineages which differentiate into mature B and T lymphocytes, respectively. Progenitors are defined by their progeny, *e.g.*, granulocyte/macrophage colony-forming progenitor cells (GM-CFU) differentiate into neutrophils or macrophages; primitive erythroid burst-forming units (BFU-E) differentiate into erythroid colony-forming units (CFU-E) which give rise to mature erythrocytes. Similarly, the Meg-CFU, Eos-CFU and Bas-CFU progenitors are able to differentiate into megakaryocytes, eosinophils and basophils, respectively.

Hematopoietic growth factors (reviewed in Andrea, *NEJM* 330(12):839-846 (1994)) have been shown to enhance growth and/or differentiation of blood cells via activation of receptors present on the surface of blood progenitor cells of the bone marrow. While some of these growth factors stimulate proliferation of restricted lineages of blood cells, others enhance proliferation of multiple lineages of blood cells. For example, erythropoietin (EPO) supports the proliferation of erythroid cells, whereas interleukin-3 (IL-3) induces proliferation of erythroid and myeloid lineages and is therefore considered a multi-lineage factor.

In recent years, several hematopoietic growth factor receptors have been isolated. Due to their low abundance and their existence in both high-affinity and low-affinity forms, biochemical characterization of these receptors has been hampered.

Cytokine receptors frequently assemble into multi-subunit complexes. Sometimes, the α subunit of this complex is involved in binding the cognate growth factor and the β-subunit may contain an ability to transduce a signal to the cell. These receptors have been assigned to three subfamilies depending on the complexes formed. Subfamily 1 includes the receptors for erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), interleukin-4 (IL-4), interleukin-7 (IL-7), growth hormone (GH) and prolactin (PRL). Ligand binding to receptors belonging to this subfamily is thought to result in homodimerization of the receptor. Subfamily 2 includes receptors for IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-5 (IL-5), interleukin-6 (IL-6), leukemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF). Subfamily 2 receptors are heterodimers having an α-subunit for ligand binding and β-subunit (either the shared β-subunit of the IL-3, GM-CSF and IL-5 receptors or the gp130 subunit of the IL-6, LIF, OSM and CNTF receptors) for signal transduction. Subfamily 3 contains only the interleukin-2 (IL-2)

receptor. The β and γ subunits of the IL-2 receptor complex are cytokine-receptor polypeptides which associate with the α-subunit of the unrelated Tac antigen.

B. OBESITY

Obesity is the most common nutritional disorder which, according to recent epidemiologic studies, affects about one third of all Americans 20 years of age or older. Kuczmarski *et al.*, *J. Am. Med. Assoc.* 272:205-11 (1994). Obesity is responsible for a variety of serious health problems, including cardiovascular disorders, type II diabetes, insulin-resistance, hypertension, hypertriglyceridemia, dyslipoproteinemia, and some forms of cancer. Pi-Sunyer, F., *Anns. Int. Med.* 119: 655-60 (1993); Colfitz, G., *Am. J. Clin. Nutr.* 55:503S-507S (1992). A single-gene mutation (the obesity or "*ob*" mutation) has been shown to result in obesity and type II diabetes in mice. Friedman, *Genomics* 11:1054-1062 (1991).

Zhang *et al.*, *Nature* 372:425-431 (1994) have recently reported the cloning and sequencing of the mouse *ob* gene and its human homologue, and suggested that the *ob* gene product, leptin or OB protein, may function as part of a signalling pathway from adipose tissue that acts to regulate the size of the body fat depot. Parabiosis experiments performed more than 20 years ago predicted that the genetically obese mouse containing two mutant copies of the *ob* gene (*ob/ob* mouse) does not produce a satiety factor which regulates its food intake, while the diabetic (*db/db*) mouse produces but does not respond to a satiety factor. Coleman and Hummal, *Am. J. Physiol.* 217:1298-1304 (1969); Coleman, *Diabetol* 9:294-98 (1973). Recent reports by three independent research teams have demonstrated that daily injections of recombinant OB protein inhibit food intake and reduce body weight and fat in grossly obese *ob/ob* mice but not in *db/db* mice (Pelleymounter *et al.*, *Science* 269:540-43 (1995); Halaas *et al.*, *Science* 269:543-46 (1995); Campfield *et al.*, *Science* 269: 546-49 (1995)), suggesting that the OB protein is such a satiety factor as proposed in early cross-circulation studies.

Researchers suggest that at least one OB receptor is localized in the brain. The identification and expression cloning of a leptin receptor (OB-R) was reported by Tartaglia *et al.* *Cell* 83:1263-71 (1995). Various isoforms of a OB receptor are described by Cioffi *et al.* *Nature* 2:585-89 (1996). See, also, WO 96/08510.

The mouse *db* gene has recently been cloned (Lee *et al.* *Nature* 379:632 (1996) and Chen *et al.* *Cell* 84:491-495 (1996)). Previous data had suggested that the *db* gene encoded the receptor for the obese (*ob*) gene product, leptin (Coleman *et al.*, *Diebetologia* 9:294-8 (1973) and Coleman *et al.*, *Diebetologia* 14:141-8 (1978)). It has been very recently confirmed that the *db/db* mouse

results from a truncated splice variant of the OB receptor which likely renders the receptor defective in signal transduction (Lee *et al.*, *Nature* 379:632 (1996) and Chen *et al.*, *Cell* 84: 491-495 (1996)).

SUMMARY OF THE INVENTION

This application relates to agonist antibodies which specifically bind to the WSX receptor and mimic one or more biological activities of naturally occurring WSX ligand, OB protein. Preferred antibodies are those with a strong binding affinity for human WSX receptor (*e.g.* having a Kd of no more than about 1 x 10^{8}M; and preferably no more than about 1 x 10^{9}M). In preferred embodiments, the agonist antibody binds to both human and murine WSX receptor.

Antibodies with defined agonistic activity in a bioassay, the KIRA ELISA, are disclosed herein. Preferred antibodies have an IC50 in the KIRA ELISA of about 0.5μg/ml or less, preferably about 0.2μg/ml or less, and most preferably about 0.1μg/ml or less.

The agonist antibodies of interest herein may have one or more of the biological characteristics of antibody 2D7, 1G4, 1E11 or 1C11 (see Example 13) or clones 3, 4, or 17 (see Example 14). For example, the antibody may bind to the epitope bound by any one of these antibodies, and/or may have some or all of the hypervariable region residues of these antibodies.

The agonist antibody may be one which decreases body weight and/or fat-depot weight and/or food intake in an obese mammal (*e.g.* in an *ob*/*ob* mouse). The preferred agonist antibody is one which exerts an adipose-reducing effect in an obese mammal (*e.g.* an *ob*/*ob* mouse) which is in excess of that induced by a reduction in food intake (Levin *et al. Proc. Natl. Acad. Sci. USA* 93:1726-1730 (1996)).

The agonist antibody may also have the property of inducing differentiation and/or proliferation and/or survival of hematopoietic progenitor cells. For example, the agonist antibody may induce lymphopoiesis, erythropoiesis and/or myelopoiesis.

The invention further provides a composition comprising the agonist antibody and a physiologically acceptable carrier. The composition for therapeutic use is sterile and may be lyophilized. For use in hematopoiesis, for example, the composition may further comprise a cytokine.

In another aspect, the invention provides a method for activating the WSX receptor which comprises exposing the WSX receptor to an amount of an agonist anti-WSX receptor antibody which is effective for activating the WSX receptor. The invention further provides a method for enhancing proliferation and/or differentiation of a cell which expresses the WSX receptor at its cell surface comprising exposing the cell to an amount of exogenous agonist anti-WSX receptor antibody which is effective for enhancing proliferation and/or differentiation of the cell. In another embodiment, the

invention provides a method for decreasing body weight and/or fat-depot weight and/or food intake in an obese mammal (*e.g.* a human) comprising administering an effective amount of the agonist antibody to the mammal. Also, the invention provides a method for treating the medical sequelae of obesity in a mammal, such as, *e.g.*, arteriosclerosis, Type II diabetes, polycystic ovarian disease, cardiovascular diseases, osteoarthritis, dermatological disorders, hypertension, insulin resistance, hypercholesterolemia, hypertriglyceridemia, cancer and cholelithiasis, comprising administering an effective amount of an agonist anti-WSX receptor antibody to the mammal. The mammal to be treated may be one diagnosed with any one or more of these diseases, or may be predisposed to these diseases.

In another aspect, the present invention pertains to the discovery herein that WSX ligands, such as obesity (OB) protein, play a role in hematopoiesis via signalling through the WSX receptor. The role of the WSX receptor-ligand signalling pathway appears to be at the level of the early hematopoietic precursor as is evident by the ability of OB protein to simulate myelopoiesis, erythropoiesis (*e.g.* splenic erythropoiesis) and most dramatically, lymphopoiesis. Accordingly, WSX ligands can be used to stimulate proliferation and/or differentiation and/or survival of hematopoietic progenitor cells either *in vitro* or *in vivo* (*e.g.* for treating hematopoietic diseases or disorders).

Thus, the invention provides a method for stimulating proliferation and/or differentiation of a cell which expresses the WSX receptor (especially the WSX receptor variant 13.2, which is demonstrated herein to have the capacity to transmit a proliferative signal) at its cell surface comprising the step of contacting the WSX receptor with an amount of WSX ligand which is effective for stimulating proliferation and/or OB protein differentiation of the cell. In prefered embodiments, the cell which is exposed to the WSX ligand is a hematopoeitic precursor, *e.g.* a CD34+ cell. The WSX ligand may be OB protein or an agonist antibody which binds to the WSX receptor. For *in vivo* use, the WSX ligand of choice may be a long half-life derivative of an OB protein, such as OB-immunoglobulin chimera and/or OB protein modified with a nonproteinaceous polymer, such as polyethylene glycol (PEG). The method contemplated herein may lead to an increase in the proliferation and/or differentiation of lymphoid, myeloid and/or erythroid blood cell lineages and encompasses both *in vitro* and *in vivo* methods. For *in vitro* uses, the cell possessing the WSX receptor may be present in cell culture. As to *in vivo* methods, the cell may be present in a mammal, especially a human (*e.g.* one who is suffering from decreased blood levels and who could benefit from an increase in various blood cells). Potential patients include those who have undergone chemo- or radiation therapy, or bone marrow transplantation therapy. Thus, the invention provides a method for repopulating blood cells (*e.g.* erythroid, myeloid and/or lymphoid blood cells) in a

mammal comprising administering to the mammal a therapeutically effective amount of a WSX ligand.

Mammals which may benefit from an enhancement of lymphopoiesis include those predisposed to, or suffering from, any ony or more of the following exemplary conditions: lymphocytopenia; lymphorrhea; lymphostasis; immunodeficiency (*e.g.* HIV and AIDS); infections (including, for example, opportunistic infections and tuberculosis (TB)); lupus; and other disorders characterized by lymphocyte deficiency. An effective amount of the WSX ligand can be used in a method of immunopotentiation or to improve immune function in a mammal.

On the other hand, WSX receptor or WSX ligand antagonists (such as WSX receptor ECD or immunoadhesin, and WSX receptor or OB protein neutralizing antibodies) may be used in the treatment of those disorders wherein unacceptable lymphocyte levels are present in the mammal, particularly where this is caused by excessive activation of the WSX receptor. Examples of conditions in which administration of such an antagonist may be beneficial include: neoplastic disorders (such as Hodkin's disease; lymphosarcoma; lymphoblastoma; lymphocytic leukemia; and lymphoma) and lymphocytosis.

Diseases or disorders in which an increase in erythropoiesis may be beneficial include, but are not limited to: erythrocytopenia; erthrodegenerative disorders; erythroblastopenia; leukoerythroblastosis; erythroclasis; thalassemia; and anemia (*e.g.* hemolytic anemia, such as acquired, autoimmune, or microangiopathic hemolytic anemia; aplastic anemia; congenital anemia, *e.g.*, congenital dyserythropoietic anemia, congenital hemolytic anemia or congenital hypoplastic anemia; dyshemopoietic anemia; Faconi's anemia; genetic anemia; hemorrhagic anemia; hyperchromic or hypochromic anemia; nutritional, hypoferric, or iron deficiency anemia; hypoplastic anemia; infectious anemia; lead anemia; local anemia; macrocytic or microcytic anemia; malignant or pernicious anemia; megaloblastic anemia; molecular anemia; normocytic anemia; physiologic anemia; traumatic or posthemorrhagic anemia; refractory anemia; radiation anemia; sickle cell anemia; splenic anemia; and toxic anemia).

Conversely, WSX receptor or WSX ligand antagonists may be used to treat those conditions in which excessive erythrocyte levels are present in a mammal, *e.g.* in neoplastic disorders such as erythroleukemia; erythroblastosis; and erythrocythemia or polycythemia.

An increase in myelopoiesis may be beneficial in any of the above-mentioned diseases or disorders as well as the following exemplary conditions: myelofibrosis; thrombocytopenia; hypoplasia; disseminated intravascular coagulation (DIC); immune (autoimmune) thrombocytopenic purpura (ITP); HIV induced ITP; myelodysplasia; thrombocytotic diseases and thrombocytosis.

Antagonists of the WSX receptor-WSX ligand interaction may also be used to treat myeloid cell-related conditions such as malignancies (*e.g.* myelosarcoma, myeloblastoma, myeloma, myeloleukemia and myelocytomatosis); myeloblastosis; myelocytosis; and myelosis.

The method may further involve the step of exposing hematopoeitic cells (whether they be in cell culture or in a mammal) to one or more other cytokines (*e.g.* lineage-specific cytokines) and this may lead to a synergistic enhancement of the proliferation and/or differentiation of the cells. Exemplary cytokines include thrombopoietin (TPO); erythropoietin (EPO); macrophage-colony stimulating factor (M-CSF); granulocyte-macrophage-CSF (GM-CSF); granulocyte-CSF (G-CSF); interleukin-1 (IL-1); IL-1α; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-11; IL10; IL-12; leukemia inhibitory factor (LIF) or kit ligand (KL). In this embodiment, exposure to the cytokine may proceed, occur simultaneously with, or follow, exposure to the WSX ligand. Preferably, the WSX ligand and one or more further cytokines are administered simultaneously to the patient (where the method is an *in vivo* one) and, optionally, are combined to form a pharmaceutical composition.

For use in the above methods, the invention also provides an article of manufacture, comprising: a container; a label on the container; and a composition comprising an active agent within the container; wherein the composition is effective for enhancing proliferation and/or differentiation of cells comprising the WSX receptor in a mammal, the label on the container indicates that the composition can be used for enhancing proliferation and/or differentiation of those cells and the active agent in the composition is a WSX ligand. Optionally, the article of manufacture includes one or more futher containers which hold further cytokine(s) in a packaged combination with the container holding the WSX ligand.

In another embodiment, an effective amount of the WSX ligand may be used to improve engraftment in bone marrow transplantation or to stimulate mobilization of hematopoietic stem cells in a mammal prior to harvesting hematopoietic progenitors from the peripheral blood thereof.

According to a further aspect, the invention is concerned with the WSX cytokine receptor and a soluble form of the receptor which is the WSX receptor extracellular domain (ECD). The WSX receptor polypeptides are optionally conjugated with, or fused to, molecules which increase the serum half-lives thereof and can be formulated as pharmaceutical compositions comprising the polypeptide and a physiologically acceptable carrier.

In certain embodiments, the WSX receptor ECD may be used as an antagonist insofar as it may bind to WSX ligand and thereby reduce activation of endogenous WSX receptor. This may be useful in conditions characterized by excess levels of WSX ligand and/or excess WSX receptor activation in a mammal. WSX receptor ECD may, for example, be used to treat metabolic disorders

(*e.g.,* anorexia or steroid-induced truncalobesity), stem cell tumors and other tumors which express WSX receptor.

Pharmaceutical compositions of the WSX receptor ECD may further include a WSX ligand. Such dual compositions may be beneficial where it is therapeutically useful to prolong the half-life of WSX ligand and/or activate endogenous WSX receptor directly as a heterotrimeric complex.

The invention also relates to chimeric WSX receptor molecules, such as WSX receptor immunoadhesins (having long half-lives in the serum of a patient treated therewith) and epitope tagged WSX receptor. Immunoadhesins may be employed as WSX receptor antagonists in conditions or disorders in which neutralization of WSX receptor biological activity may be beneficial. Bispecific immunoadhesins (combining a WSX receptor ECD with a domain of another cytokine receptor) may form high affinity binding complexes for WSX ligand.

The invention further provides methods for identifying a molecule which binds to and/or activates the WSX receptor. This is useful for discovering molecules (such as peptides, antibodies, and small molecules) which are agonists or antagonists of the WSX receptor. Such methods generally involve exposing an immobilized WSX receptor to a molecule suspected of binding thereto and determining binding of the molecule to the immobilized WSX receptor and/or evaluating whether or not the molecule activates (or blocks activation of) the WSX receptor. In order to identify such WSX ligands, the WSX receptor may be expressed on the surface of a cell and used to screen libraries of synthetic compounds and naturally occurring compounds (*e.g.,*endogenous sources of such naturally occurring compounds, such as serum). The WSX receptor can also be used as a diagnostic tool for measuring serum levels of endogenous WSX ligand.

In a further embodiment, a method for purifying a molecule which binds to the WSX receptor is provided. This can be used in the commercial production and purification of therapeutically active molecules which bind to this receptor. In the method, the molecule of interest (generally a composition comprising one or more contaminants) is adsorbed to immobilized WSX receptor (*e.g.,*WSX receptor immunoadhesin immobilized on a protein A column). The contaminants, by virtue of their inability to bind to the WSX receptor, will generally flow through the column. Accordingly, it is then possible to recover the molecule of interest from the column by changing the elution conditions, such that the molecule no longer binds to the immobilized receptor.

In further embodiments, the invention provides antibodies that specifically bind to the WSX receptor. Preferred antibodies are monoclonal antibodies which are non-immunogenic in a human and bind to an epitope in the extracellular domain of the receptor. Preferred antibodies bind the WSX receptor with an affinity of at least about 10^6 L/mole, more preferably 10^7 L/mole.

Antibodies which bind to the WSX receptor may optionally be fused to a heterologous polypeptide and the antibody or fusion thereof may be used to isolate and purify WSX receptor from a source of the receptor.

In a further aspect, the invention provides a method for detecting the WSX receptor *in vitro* or *in vivo* comprising contacting the antibody with a sample suspected of containing the receptor and detecting if binding has occurred. Based on the observation herein that CD34+ cells possess WSX receptor, use of WSX antibodies for identification and/or enrichment of stem cell populations (in a similar manner to that in which CD34 antibodies are presently used) is envisaged.

For certain applications, it is desirable to have an agonist antibody which can be screened for as described herein. Such agonist antibodies are useful for activating the WSX receptor for *in vitro* uses whereby enhancement of proliferation and/or differentiation of a cell comprising the receptor is desired. Furthermore, these antibodies may be used to treat conditions in which an effective amount of WSX receptor activation leads to a therapeutic benefit in the mammal treated therewith. For example, the agonist antibody can be used to enhance survival, proliferation and/or differentiation of a cell comprising the WSX receptor. In particular, agonist antibodies and other WSX ligands may be used to stimulate proliferation of stem cells/progenitor cells either *in vitro* or *in vivo*. Other potential therapeutic applications include the use of agonist antibodies to treat metabolic disorders (such as obesity and diabetes) and to promote kidney, liver or lung growth and/or repair (*e.g.*,in renal failure).

For therapeutic applications it is desirable to prepare a composition comprising the agonist antibody and a physiologically acceptable carrier. Optionally, such a composition may further comprise one or more cytokines.

In other embodiments, the antibody is a neutralizing antibody. Such molecules can be used to treat conditions characterized by unwanted or excessive activation of the WSX receptor.

In addition to the above, the invention provides isolated nucleic acid molecules, expression vectors and host cells encoding the WSX receptor which can be used in the recombinant production of WSX receptor as described herein. The isolated nucleic acid molecules and vectors are also useful for gene therapy applications to treat patients with WSX receptor defects and/or to increase responsiveness of cells to WSX ligand.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-H together depict the double stranded nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) encoding full length human WSX receptor variant 13.2. Nucleotides are numbered at the beginning of the sense strand. Amino acid residues are numbered

at the beginning of the amino acid sequence. Restriction enzyme sites are depicted above the nucleotide sequence.

Figs. 2A-B together depict an amino acid sequence alignment of full length human WSX receptor variants 6.4 (SEQ ID NO:3), 12.1 (SEQ ID NO:4) and 13.2, respectively. Homologous residues are boxed. WSX receptor variants 6.4, 12.1 and 13.2 are native sequence human WSX receptor variants which, without being bound to any one theory, appear to be generated by alternate splicing of WSX receptor mRNA. The putative signal peptide, transmembrane, Box 1, Box 2, and Box 3 domains are indicated. The extracellular and cytoplasmic domains are amino- and carboxy-terminal, respectively, to the transmembrane domain. The Box 1-3 domains shown correspond to the box 1-3 motifs described in Baumann *et al., Mol. Cell. Biol.* 14(1):138-146 (1994).

Figs. 3A-E together depict an alignment of the nucleotide sequences encoding human WSX receptor variants 6.4 (SEQ ID NO:5), 12.1 (SEQ ID NO:6) and 13.2, respectively.

Figs. 4A-B depict an alignment of the full length human WSX receptor variant 13.2 amino acid sequence (top) with that of partial murine WSX receptor extracellular domain sequence (bottom) (SEQ ID NO:7) obtained as described in Example 7. The putative murine signal peptide is marked with an arrow.

Figs. 5A-F represent an alignment of the nucleotide sequences encoding human WSX receptor variant 13.2 (bottom) and partial murine WSX receptor extracellular domain (top) (SEQ ID NO:8), respectively.

Fig. 6 is a bar graph depicting results of the thymidine incorporation assay described in Example 5. ^{3}H-thymidine incorporation (counts per minute, CPM) in parental Baf3 cells or Baf3 cells electroporated with GH/WSX variant 13.2 chimera in the presence of varying concentrations of human growth hormone (GH) is shown.

Fig. 7 shows the human and murine oligonucleotides (SEQ ID NOS:9-38, respectively) used for the antisense experiment described in Example 8.

Figs. 8 and 9 show thymidine incorporation assays in Baf-3 cells. For these assays, cells were deprived of IL-3 for 16-18 hours (in RPMI 1640 supplemented with 10% fetal calf serum (FCS)). Cells were washed in serum free RPMI 1640 and plated at 50,000 cells per well in 0.2 mls of serum free RPMI 1640 supplemented with the indicated concentration of human GH or human OB protein. Cells were stimulated for 24 hours and thymidine incorporation was determined as described (Zeigler *et al. Blood* 84:2422-2430 (1994)). Assays were performed in triplicate and the results were confirmed in three independent experiments.

In Fig. 8, GH receptor-WSX receptor variant 12.1 or 13.2 chimeric proteins were expressed in Baf-3 cells as described in Example 5. These transfected cells and the parental Baf-3 line were stimulated with hGH and the incorporation of titrated thymidine determined.

In Fig. 9, Baf-3 cells were stably transfected with WSX receptor variant 13.2. Thymidine incorporation was then determined in these cell lines following stimulation with human OB protein.

In Figs. 10A-C, murine fetal liver AA4$^+$Sca$^+$Kit$^+$ (flASK) stem cells were cultured in suspension culture or methylcellulose. In Fig. 10A, flASK cells were cultured in suspension culture containing serum with kit ligand (KL) or kit ligand and OB protein. Cell counts and cytospin analyses were performed 7 days later. In Fig. 10B, flASK cells were seeded into methylcellulose under either myeloid or lymphoid conditions as described in Example 10. Colony counts were performed 14 days later. For colonies produced under lymphoid conditions, FACS analysis demonstrated the vast majority of cells to be B220 positive. In Fig. 10C, flASK cells were seeded into methylcellulose containing kit ligand. To this base media, erythropoietin (EPO) or erythropoietin and OB protein were then added. The resultant colonies were counted 14 days later. FACS analysis demonstrated approximately 95% of these colonies to be TER 119 positive. All assays were performed in triplicate and confirmed in at least three independent experiments.

Fig. 11 illustrates methylcellulose assays to determine the colony forming potential of *db/db*, *ob/ob* and the corresponding wild-type marrow. 100,000 bone marrow cells were seeded into methylcellulose and the resultant colonies counted after 14 days. Assays were performed using both myeloid and lymphoid conditions. Assays were performed in triplicate and the experiments were repeated a minimum of 3 times.

Figs. 12A-B show bone marrow cellular profiles in wild-type misty gray homozygotes, misty gray/*db* heterozygotes, and homozygote *db/db* mice. Overall cellularity in the *db/db* marrow was unchanged compared to controls. Fig. 12A shows cellular profiles determined using anti-B220, anti-CD43, and anti-TER119 antibodies. Fig. 12B shows cellular profiles of the spleens from the above groups.

Figs. 13A-C are an analysis of peripheral blood in *db/db* homozygotes, *db/db* misty gray heterozygotes and misty gray homozygotes. 40 microliters of peripheral blood was taken via orbital bleed and analyzed on a Serrono Baker system 9018. All areas described by the boxes represent the mean ± one standard deviation of the two parameters.

Fig. 14 is a comparison of peripheral lymphocyte counts and blood glucose level. Five groups of animals, misty-gray, misty-gray/*db*, *db/db*, interferon α-transgenic, and glucokinase transgenic heterozygote mice (gLKa) were sampled via retro-orbital bleed. Blood glucose levels in

these mice were determined. All areas described by the boxes represent the mean ± standard deviation of the two parameters.

In Figs. 15A-C, misty gray homozygotes, *db*/misty gray heterozygotes, and homozygous *db/db* mice were subjected to sub-lethal irradiation and the recovery kinetics of the peripheral blood was determined via retro-orbital bleeds.

Figs. 16A-16Q together show the nucleotide sequence (SEQ ID NO:46) and the amino acid sequence (SEQ ID NO: 47) of the human OB-immunoglobulin chimera in the plasmid described in of Example 11.

Fig. 17 shows binding of anti-WSX receptor agonist antibodies to human WSX receptor. The anti-WSX receptor agonist antibodies (2D7 and 1G4) produced as described in Example 13 and an IgG isotope control were evaluated for their ability to bind to human WSX receptor by capture ELISA.

Fig. 18 shows the activity of mAbs 2D7 and 1G4 as well as OB protein in the KIRA ELISA (see Example 13). Absorbance at 490nm versus concentration of antibody or ligand in this assay is shown.

Fig. 19 depicts binding of anti-WSX receptor agonist antibodies to murine WSX receptor. The anti-WSX receptor agonist antibodies (2D7 and 1G4) and an IgG isotope control were evaluated for their ability to bind to murine WSX receptor by capture ELISA.

Figs. 20A-B show the results of epitope mapping of the agonist anti-WSX receptor antibodies produced as described in Example 13. Fig. 20A shows blocking ability of anti-WSX receptor antibodies on Epitope A using biotinylated 2D7. Fig. 20B shows blocking ability of anti-WSX receptor antibodies on Epitope B using biotinylated 1C11. Based on the competitive binding ELISA, 2D7 bound a different epitope from 1E11, 1C11 and 1G4.

Fig. 21 depicts an alignment of the amino acid sequences of full length human WSX receptor variant 6.4 (hWSXR) (SEQ ID NO:3) and murine WSX receptor (mWSXR) (SEQ ID NO:51).

Fig. 22 is a standard curve for human OB protein in the KIRA ELISA, which illustrates schematically inside the graph WSX receptor KIRA ELISA panning with scFv phage as described in Example 14.

Fig. 23 shows the activity of clone # 3, #4 and # 17 scFv phage from Example 14 and anti-HER2 scFv phage control in the KIRA ELISA. Absorbance versus phage titer is shown.

Fig. 24 shows the activity of clone # 3, #4 and # 17 scFv from Example 14, anti-HER2 scFv control (Her2 clone) and OB protein in the KIRA ELISA. Absorbance versus antibody concentration is shown.

Fig. 25 aligns the amino acid sequences of agonist antibody clone #3 (3.scFv) (SEQ ID NO:48), clone #4 (4.scFv) (SEQ ID NO:49) and clone #17 (17.scFv) (SEQ ID NO:50) obtained as

described in Example 14. Complementarity determining region (CDR) residues according to Kabat *et al., Sequences of Proteins of Immunological Interest.* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991) are underlined and hypervariable loop (Chothia *et al., Nature* 342:8767 (1989)) are in italics.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

The terms "WSX receptor" or "WSX receptor polypeptide" when used herein encompass native sequence WSX receptor; WSX receptor variants; WSX extracellular domain; and chimeric WSX receptor (each of which is defined herein). Optionally, the WSX receptor is not associated with native glycosylation. "Native glycosylation" refers to the carbohydrate moieties which are covalently attached to WSX receptor when it is produced in the mammalian cell from which it is derived in nature. Accordingly, human WSX receptor produced in a non-human cell is an example of a WSX receptor which is "not associated with native glycosylation". Sometimes, the WSX receptor is unglycosylated (*e.g.,*as a result of being produced recombinantly in a prokaryote).

"WSX ligand" is a molecule which binds to and activates native sequence WSX receptor (especially WSX receptor variant 13.2). The ability of a molecule to bind to WSX receptor can be determined by the ability of a putative WSX ligand to bind to WSX receptor immunoadhesin (see Example 2) coated on an assay plate, for example. The thymidine incorporation assay provides a means for screening for WSX ligands which activate the WSX receptor. Exemplary WSX ligands include anti-WSX receptor agonist antibodies and OB protein (*e.g.*, described in Zhang *et al. Nature* 372:425-431 (1994)).

The terms "OB protein" and "OB" are used interchangeably herein and refer to native sequence OB proteins (also known as "leptins") and their functional derivatives.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (*e.g.*, WSX receptor or OB protein) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "native sequence WSX receptor" specifically encompasses naturally-occurring truncated forms of the WSX receptor, naturally-occurring variant forms (*e.g.,*alternatively spliced

forms such as human WSX receptor variants 6.4, 12.1 and 13.2 described herein) and naturally-occurring allelic variants of the WSX receptor. The preferred native sequence WSX receptor is a mature native sequence human WSX receptor, such as human WSX receptor variant 6.4, human WSX receptor variant 12.1 or human WSX receptor variant 13.2 (each shown in Figs. 2A-B). Most preferred is mature human WSX receptor variant 13.2.

The term "native sequence OB protein" includes those OB proteins from any animal species (*e.g.* human, murine, rabbit, cat, cow, sheep, chicken, porcine, equine, etc.) as occurring in nature. The definition specifically includes variants with or without a glutamine at amino acid position 49, using the amino acid numbering of Zhang *et al.*, *supra*. The term "native sequence OB protein" includes the native proteins with or without the initiating N-terminal methionine (Met), and with or without the native signal sequence, either in monomeric or in dimeric form. The native sequence human and murine OB proteins known in the art are 167 amino acids long, contain two conserved cysteines, and have the features of a secreted protein. The protein is largely hydrophilic, and the predicted signal sequence cleavage site is at position 21, using the amino acid numbering of Zhang *et al.*, *supra*. The overall sequence homology of the human and murine sequences is about 84%. The two proteins show a more extensive identity in the N-terminal region of the mature protein, with only four conservative and three non-conservative substitutions among the residues between the signal sequence cleavage site and the conserved Cys at position 117. The molecular weight of OB protein is about 16 kD in a monomeric form.

The "WSX receptor extracellular domain" (ECD) is a form of the WSX receptor which is essentially free of the transmembrane and cytoplasmic domains of WSX receptor, *i.e.,* has less than 1% of such domains, preferably 0.5 to 0% of such domains, and more preferably 0.1 to 0% of such domains. Ordinarily, the WSX receptor ECD will have an amino acid sequence having at least about 95% amino acid sequence identity with the amino acid sequence of the ECD of WSX receptor indicated in Figs. 2A-B for human WSX receptor variants 6.4, 12.1 and 13.2, preferably at least about 98%, more preferably at least about 99% amino acid sequence identity, and thus includes WSX receptor variants as defined below.

A "variant" polypeptide means a biologically active polypeptide as defined below having less than 100% sequence identity with a native sequence polypeptide (*e.g.*, WSX receptor having the deduced amino acid sequence shown in Figs. 1A-H for human WSX receptor variant 13.2). Such variants include polypeptides wherein one or more amino acid residues are added at the N- or C-terminus of, or within, the native sequence; from about one to thirty amino acid residues are deleted, and optionally substituted by one or more amino acid residues; and derivatives of the above polypeptides, wherein an amino acid residue has been covalently modified so that the resulting

product has a non-naturally occurring amino acid. Ordinarily, a biologically active WSX receptor variant will have an amino acid sequence having at least about 90% amino acid sequence identity with human WSX receptor variant 13.2 shown in Figs. 1A-H, preferably at least about 95%, more preferably at least about 99%. Ordinarily, a biologically active OB protein variant will have an amino acid sequence having at least about 90% amino acid sequence identity with a native sequence OB protein, preferably at least about 95%, more preferably at least about 99%.

A "chimeric" OB protein or WSX receptor is a polypeptide comprising OB protein or full-length WSX receptor or one or more domains thereof (*e.g.,*the extracellular domain of the WSX receptor) fused or bonded to heterologous polypeptide. The chimeric WSX receptor will generally share at least one biological property in common with human WSX receptor variant 13.2. The chimeric OB protein will generally share at least one biological property in common with a native sequence OB protein. Examples of chimeric polypeptides include immunoadhesins and epitope tagged polyeptides.

The term "WSX immunoadhesin" is used interchangeably with the expression "WSX receptor-immunoglobulin chimera" and refers to a chimeric molecule that combines a portion of the WSX receptor (generally the extracellular domain thereof) with an immunoglobulin sequence. Likewise, an “OB protein immunoadhesin” or “OB-immunoglobulin chimera” refers to a chimeric molecule which combines OB protein (or a portion thereof) with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD or IgM, but preferably IgG1 or IgG3.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising WSX receptor or OB protein fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody thereagainst can be made, yet is short enough such that it does not interfere with biological activity of the WSX receptor or OB protein. The tag polypeptide preferably also is fairly unique so that the antibody thereagainst does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues).

"Isolated" WSX receptor (or OB protein) means WSX receptor (or OB protein) that has been purified from a WSX receptor (or OB protein) source or has been prepared by recombinant or synthetic methods and is sufficiently free of other peptides or proteins (1) to obtain at least 15 and preferably 20 amino acid residues of the N-terminal or of an internal amino acid sequence by using a spinning cup sequenator or the best commercially available amino acid sequenator marketed or

as modified by published methods as of the filing date of this application, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Homogeneity here means less than about 5% contamination with other source proteins.

"Essentially pure" protein means a composition comprising at least about 90% by weight of the protein, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" protein means a composition comprising at least about 99% by weight of protein, based on total weight of the composition.

"Biological property" when used in conjunction with either "WSX receptor" or "isolated WSX receptor" means having an effector or antigenic function or activity that is directly or indirectly caused or performed by native sequence WSX receptor (whether in its native or denatured conformation). Effector functions include ligand binding; and enhancement of survival, differentiation and/or proliferation of cells (especially proliferation of cells). However, effector functions do not include possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against native sequence WSX receptor.

"Biological property" when used in conjunction with either "OB protein" or "isolated OB protein" means having an effector function that is directly or indirectly caused or performed by native sequence OB protein. Effector functions of native sequence OB protein include WSX receptor binding and activation; and enhancement of differentiation and/or proliferation of cells expressing this receptor (as determined in the thymidine incorporation assay, for example). A "biologically active" OB protein is one which possesses a biological property of native sequence OB protein.

A "functional derivative" of a native sequence OB protein is a compound having a qualitative biological property in common with a native sequence OB protein. "Functional derivatives" include, but are not limited to, fragments of native sequence OB proteins and derivatives of native sequence OB proteins and their fragments, provided that they have a biological activity in common with a corresponding native sequence OB protein. The term "derivative" encompasses both amino acid sequence variants of OB protein and covalent modifications thereof.

The phrase "long half-life" as used in connection with OB derivatives, concerns OB derivatives having a longer plasma half-life and/or slower clearance than a corresponding native sequence OB protein. The long half-life derivatives preferably will have a half-life at least about 1.5-times longer than a native OB protein; more preferably at least about 2-times longer than a native OB protein, more preferably at least about 3-time longer than a native OB protein. The native OB protein preferably is that of the individual to be treated.

An "antigenic function" means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against native sequence WSX receptor. The principal antigenic

function of a WSX receptor is that it binds with an affinity of at least about 10^6 L/mole to an antibody raised against native sequence WSX receptor. Ordinarily, the polypeptide binds with an affinity of at least about 10^7 L/mole. The antibodies used to define "antigenic function" are rabbit polyclonal antibodies raised by formulating the WSX receptor in Freund's complete adjuvant, subcutaneously injecting the formulation, and boosting the immune response by intraperitoneal injection of the formulation until the titer of the anti-WSX receptor or antibody plateaus.

"Biologically active" when used in conjunction with either "WSX receptor" or "isolated WSX receptor" means a WSX receptor polypeptide that exhibits or shares an effector function of native sequence WSX receptor and that may (but need not) in addition possess an antigenic function. A principal effector function of the WSX receptor is its ability to induce proliferation of CD34+ human umbilical cord blood cells in the colony assay described in Example 8.

"Antigenically active" WSX receptor is defined as a polypeptide that possesses an antigenic function of WSX receptor and that may (but need not) in addition possess an effector function.

"Percent amino acid sequence identity" is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the native sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the candidate sequence shall be construed as affecting sequence identity or homology.

A "thymidine incorporation assay" can be used to screen for molecules which activate the WSX receptor. In order to perform this assay, IL-3 dependent Baf3 cells (Palacios *et al., Cell*, 41:727-734 (1985)) are stably transfected with full length native sequence WSX receptor as described in Example 4. The WSX receptor/Baf3 cells so generated are starved of IL-3 for, *e.g.*, 24 hours in a humidified incubator at 37°C in 5%CO_2 and air. Following IL-3 starvation, the cells are plated out in 96 well culture dishes with, or without, a test sample containing a potential agonist (such test samples are optionally diluted) and cultured for 24 hours in a cell culture incubator. 20µl of serum free RPMI media containing 1µCi of ^{3}H thymidine is added to each well for the last 6-8 hours. The cells are then harvested in 96 well filter plates and washed with water. The filters are then counted using a Packard Top Count Microplate Scintillation Counter, for example. Agonists are expected to induce a statistically significant increase (to a P value of 0.05) in ^{3}H uptake, relative to control. Preferred agonists leads to an increase in ^{3}H uptake which is at least two fold of that of the control.

An "isolated" WSX receptor nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily

associated in the natural source of the WSX receptor nucleic acid. An isolated WSX receptor nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated WSX receptor nucleic acid molecules therefore are distinguished from the WSX receptor nucleic acid molecule as it exists in natural cells. However, an isolated WSX receptor nucleic acid molecule includes WSX receptor nucleic acid molecules contained in cells that ordinarily express WSX receptor where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies, antibody compositions with polyepitopic specificity, bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired biological activity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567 (Cabilly *et al.*)). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly *et al.*, *supra*; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient

antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). The humanized antibody includes a Primatized™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"Non-immunogenic in a human" means that upon contacting the polypeptide of interest in a physiologically acceptable carrier and in a therapeutically effective amount with the appropriate tissue of a human, no state of sensitivity or resistance to the polypeptide of interest is demonstrable upon the second administration of the polypeptide of interest after an appropriate latent period (*e.g.*, 8 to 14 days).

By "agonist antibody" is meant an antibody which is able to activate native sequence WSX receptor. The agonist antibody of particular interest herein is one which mimics one or more (*e.g.* all) of the biological properties of naturally occurring WSX ligand, OB protein. In preferred embodiments, the agonist antibody has a quantitative biological property of OB protein which is within about two orders of magnitude, and preferably within about one order of magnitude, that of OB protein. The agonist antibody may bind to and activate WSX receptor and thereby stimulate proliferation and/or differentiation and/or maturation and/or survival of a cell which expresses the

WSX receptor (*e.g.* WSX receptor variant 13.2). In this embodiment of the invention, the agonist antibody may be one which enhances proliferation and/or differentiation of a hematopoietic progenitor cell which expresses the WSX receptor at its cell surface; enhances proliferation and/or differentiation of lymphoid blood cell lineages; enhances proliferation and/or differentiation of myeloid blood cell lineages; and/or enhances proliferation and/or differentiation of erythroid blood cell lineages. The agonist antibody may display agonist activity upon binding to a chimeric receptor comprising the WSX receptor extracellular domain in the KIRA ELISA. The agonist antibody may stimulate ^{3}H uptake in the thymidine incorporation assay using a signaling WSX receptor (see above); decrease body weight and/or fat-depot weight and/or food intake in an obese mammal (*e.g.* in the *ob/ob* mouse); effect Ca^{2+} influx in adipocytes; and/or activate downstream signaling molecules of OB protein.

A "neutralizing antibody" is one which is able to block or significantly reduce an effector function of native sequence WSX receptor or OB protein. For example, a neutralizing antibody may inhibit or reduce WSX receptor activation by a WSX ligand as determined in the thymidine incorporation assay or in a KIRA ELISA.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.,* I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Taxotere (docetaxel), Busulfan, Cytoxin, Taxol, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincreistine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan and other related nitrogen mustards.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, *e.g.,* Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella *et al.,* "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt *et al.,* (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-

amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

An "antagonist" of the WSX receptor and/or OB protein is a molecule which prevents, or interferes with, binding and/or activation of the WSX receptor or OB protein. Such molecules can be screened for their ability to competitively inhibit WSX receptor activation by OB protein in the thymidine incorporation assay disclosed herein, for example. Examples of such molecules include: WSX receptor ECD; WSX receptor immunoadhesin; neutralizing antibodies against WSX receptor or OB protein; small molecule and peptide antagonists; and antisense nucleotides against the WSX receptor or *ob* gene.

The phrase "enhancing proliferation of a cell" encompasses the step of increasing the extent of growth and/or reproduction of the cell relative to an untreated cell either *in vitro* or *in vivo*. An increase in cell proliferation in cell culture can be detected by counting the number of cells before and after exposure to a molecule of interest. The extent of proliferation can be quantified via microscopic examination of the degree of confluency. Cell proliferation can also be quantified using the thymidine incorporation assay described herein.

By "enhancing differentiation of a cell" is meant the act of increasing the extent of the acquisition or possession of one or more characteristics or functions which differ from that of the original cell (*i.e.* cell specialization). This can be detected by screening for a change in the phenotype of the cell (*e.g.,*identifying morphological changes in the cell).

A "hematopoietic progenitor cell" or "primitive hematopoietic cell" is one which is able to differentiate to form a more committed or mature blood cell type.

"Lymphoid blood cell lineages" are those hematopoietic precursor cells which are able to differentiate to form lymphocytes (B-cells or T-cells). Likewise, "lymphopoeisis" is the formation of lymphocytes.

"Erythroid blood cell lineages" are those hematopoietic precursor cells which are able to differentiate to form erythrocytes (red blood cells) and "erythropoeisis" is the formation of erythrocytes.

The phrase "myeloid blood cell lineages", for the purposes herein, encompasses all hematopoietic precursor cells, other than lymphoid and erythroid blood cell lineages as defined

above, and "myelopoiesis" involves the formation of blood cells (other than lymphocytes and erythrocytes).

A "CD34+ cell population" is enriched for hematopoietic stem cells. A CD34+ cell population can be obtained from umbilical cord blood or bone marrow, for example. Human umbilical cord blood CD34+ cells can be selected for using immunomagnetic beads sold by Miltenyi (California), following the manufacturer's directions.

"Physiologically acceptable" carriers, excipients, or stabilizers are ones which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.,* IgG1, IgG2, IgG3, and IgG4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule. Exemplary salvage receptor binding epitope sequences include HQNLSDGK (SEQ ID NO:39); HQNISDGK (SEQ ID NO:40); HQSLGTQ (SEQ ID NO:41); VISSHLGQ (SEQ ID NO:42); and PKNSSMISNTP (SEQ ID NO:43).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are OB protein; growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-

CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; and other polypeptide factors including leukemia inhibitory factor (LIF) and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

A "lineage-specific cytokine" is one which acts on relatively committed cells in the hematopoietic cascade and gives rise to an expansion in blood cells of a single lineage. Examples of such cytokines include EPO, TPO, and G-CSF.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

The term "obesity" is used to designate a condition of being overweight associated with excessive bodily fat. The desirable weight for a certain individual depends on a number of factors including sex, height, age, overall built, etc. The same factors will determine when an individual is considered obese. The determination of an optimum body weight for a given individual is well within the skill of an ordinary physician.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

By "solid phase" is meant a non-aqueous matrix to which a reagent of interest (*e.g.,*the WSX receptor or an antibody thereto) can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.,*controlled pore glass), polysaccharides (*e.g.,*agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.,*an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

II. Modes for Carrying Out the Invention

The present invention is based on the discovery of the WSX receptor. The experiments described herein demonstrate that this molecule is a cytokine receptor which appears to play a role in enhancing proliferation and/or differentiation of hematopoietic cells. In particular, this receptor has been found to be present in enriched human stem cell populations, thus indicating that WSX ligands, such as agonist antibodies, may be used to stimulate proliferation of hematopoietic stem cells/progenitor cells. Other uses for this receptor will be apparent from the following discussion.

A description follows as to how WSX receptor or OB proteins may be prepared.

A. Preparation of WSX Receptor or OB Protein

Techniques suitable for the production of WSX receptor or OB protein are well known in the art and include isolating WSX receptor or OB protein from an endogenous source of the polypeptide, peptide synthesis (using a peptide synthesizer) and recombinant techniques (or any combination of these techniques). The preferred technique for production of WSX receptor or OB protein is a recombinant technique to be described below.

Most of the discussion below pertains to recombinant production of WSX receptor or OB protein by culturing cells transformed with a vector containing WSX receptor or OB protein nucleic acid and recovering the polypeptide from the cell culture. It is further envisioned that the WSX receptor or OB protein of this invention may be produced by homologous recombination, as provided for in WO 91/06667, published 16 May 1991.

Briefly, this method involves transforming primary human cells containing a WSX receptor or OB protein-encoding gene with a construct (*i.e.*, vector) comprising an amplifiable gene (such as dihydrofolate reductase (DHFR) or others discussed below) and at least one flanking region of a length of at least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of the WSX receptor or OB protein gene to provide amplification of the WSX receptor or OB protein gene. The amplifiable gene must be at a site that does not interfere with expression of the WSX receptor or OB protein gene. The transformation is conducted such that the construct becomes homologously integrated into the genome of the primary cells to define an amplifiable region.

Primary cells comprising the construct are then selected for by means of the amplifiable gene or other marker present in the construct. The presence of the marker gene establishes the presence and integration of the construct into the host genome. No further selection of the primary cells need be made, since selection will be made in the second host. If desired, the occurrence of the homologous recombination event can be determined by employing PCR and either sequencing the resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when DNA from correct homologous integrants is present and expanding only those cells containing such fragments. Also if desired, the selected cells may be amplified at this point by stressing the cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the amplification step is not conducted until after the second transformation described below.

After the selection step, DNA portions of the genome, sufficiently large to include the entire amplifiable region, are isolated from the selected primary cells. Secondary mammalian expression host cells are then transformed with these genomic DNA portions and cloned, and clones are

selected that contain the amplifiable region. The amplifiable region is then amplified by means of an amplifying agent if not already amplified in the primary cells. Finally, the secondary expression host cells now comprising multiple copies of the amplifiable region containing WSX receptor or OB protein are grown so as to express the gene and produce the protein.

1. Isolation of DNA Encoding WSX Receptor or OB Protein

The DNA encoding WSX receptor or OB protein may be obtained from any cDNA library prepared from tissue believed to possess the WSX receptor or OB protein mRNA and to express it at a detectable level. Accordingly, WSX receptor or OB protein DNA can be conveniently obtained from a cDNA library prepared from mammalian fetal liver. The WSX receptor or OB protein-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries are screened with probes (such as antibodies to the WSX receptor or OB protein, or oligonucleotides of about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding WSX receptor or OB protein is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various human tissues, preferably human fetal liver. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide must be labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P-labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Amino acid sequence variants of WSX receptor or OB protein are prepared by introducing appropriate nucleotide changes into the WSX receptor or OB protein DNA, or by synthesis of the desired WSX receptor or OB protein. Such variants represent insertions, substitutions, and/or specified deletions of, residues within or at one or both of the ends of the amino acid sequence of a naturally occurring human WSX receptor or OB protein, such as the WSX receptor variants shown in Figs 2A-B or the human OB protein of Zhang *et al.*, *supra*. Preferably, these variants represent insertions and/or substitutions within or at one or both ends of the mature sequence, and/or insertions, substitutions and/or specificed deletions within or at one or both of the ends of the signal

sequence of the WSX receptor or OB protein. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final construct, provided that the final construct possesses the desired biological activity as defined herein. The amino acid changes also may alter post-translational processes of the WSX receptor or OB protein, such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, and/or altering the intracellular location of the WSX receptor or OB protein by inserting, deleting, or otherwise affecting the leader sequence of the WSX receptor or OB protein.

Variations in the native sequence as described above can be made using any of the techniques and guidelines for conservative and non-conservative mutations set forth in U.S. Pat. No. 5,364,934. These include oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. See also, for example, Table I therein and the discussion surrounding this table for guidance on selecting amino acids to change, add, or delete.

2. Insertion of Nucleic Acid into Replicable Vector

The nucleic acid (*e.g.,* cDNA or genomic DNA) encoding the WSX receptor or OB protein is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

a. Signal sequence component

The WSX receptor or OB proteins of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the WSX receptor or OB protein DNA that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native WSX receptor or OB protein signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.,* the yeast invertase leader, α factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In

mammalian cell expression the native signal sequence (*e.g.,* the WSX receptor or OB protein presequence that normally directs secretion of WSX receptor or OB protein from human cells *in vivo*) is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from other animal WSX receptors or OB proteins, and signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal.

The DNA for such precursor region is ligated in reading frame to DNA encoding the mature WSX receptor or OB protein.

b. *Origin of replication component*

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of WSX receptor or OB protein DNA. However, the recovery of genomic DNA encoding WSX receptor or OB protein is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the WSX receptor or OB protein DNA.

c. *Selection gene component*

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells

grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.,* ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.,* the gene encoding D-alanine racemase for *Bacilli.*

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the WSX receptor or OB protein nucleic acid, such as DHFR or thymidine kinase. The mammalian cell transformants are placed under selection pressure that only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes WSX receptor or OB protein. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of WSX receptor or OB protein are synthesized from the amplified DNA. Other examples of amplifiable genes include metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al., Proc. Natl. Acad. Sci. USA* 77:4216 (1980). The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding WSX receptor or OB protein. This amplification technique can be used with any otherwise suitable host, *e.g.,* ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding WSX receptor or OB protein, wild-type

DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.,* kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature* 282:39 (1979)). The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics* 85:12 (1977). The presence of the *trp*1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu*2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu*2 gene.

In addition, vectors derived from the 1.6 µm circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Bianchi *et al.*, *Curr. Genet.* 12:185 (1987). More recently, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology* 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer *et al.*, *Bio/Technology* 9:968-975 (1991).

d. *Promoter Component*

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the WSX receptor or OB protein nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence, such as the WSX receptor or OB protein nucleic acid sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, *e.g.,* the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to WSX receptor or OB protein-encoding DNA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native WSX receptor or OB protein promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the WSX receptor or OB protein DNA. However, heterologous promoters are

preferred, as they generally permit greater transcription and higher yields of WSX receptor or OB protein as compared to the native WSX receptor or OB protein promoter.

Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems (Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281:544 (1979)), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776), and hybrid promoters such as the tac promoter. deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled worker operably to ligate them to DNA encoding WSX receptor or OB protein (Siebenlist *et al.*, *Cell* 20:269 (1980)) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding WSX receptor or OB protein.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, *J. Biol. Chem.* 255:2073 (1980)) or other glycolytic enzymes (Hess *et al.*, *J. Adv. Enzyme Reg.* 7:149 (1968); Holland, *Biochemistry* 17:4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

WSX receptor or OB protein transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an

immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the WSX receptor or OB protein sequence, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, *Nature* 273:113 (1978); Mulligan *et al., Science* 209:1422-1427 (1980); Pavlakis *et al.*, *Proc. Natl. Acad. Sci. USA* 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. Greenaway *et al.*, *Gene* 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. See also Gray *et al.*, *Nature* 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus; Canaani *et al.*, *Proc. Natl. Acad. Sci. USA* 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells; and Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

e. *Enhancer element component*

Transcription of a DNA encoding the WSX receptor or OB protein of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent, having been found 5' (Laimins *et al.*, *Proc. Natl. Acad. Sci. USA* 78:993 (1981)) and 3' (Lusky *et al.*, *Mol. Cell Bio.* 3:1108 (1983)) to the transcription unit, within an intron (Banerji *et al., Cell* 33:729 (1983)), as well as within the coding sequence itself. Osborne *et al.*, *Mol. Cell Bio.* 4:1293 (1984). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the WSX receptor or OB protein-encoding sequence, but is preferably located at a site 5' from the promoter.

f. ***Transcription termination component***

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding WSX receptor or OB protein.

g. ***Construction and analysis of vectors***

Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, *Nucleic Acids Res.* 9:309 (1981) or by the method of Maxam *et al.*, *Methods in Enzymology* 65:499 (1980).

h. ***Transient expression vectors***

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding WSX receptor or OB protein. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Sambrook *et al.*, *supra*, pp. 16.17 - 16.22. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention for purposes of identifying analogs and variants of WSX receptor or OB protein that are biologically active WSX receptor or OB protein.

i. ***Suitable exemplary vertebrate cell vectors***

Other methods, vectors, and host cells suitable for adaptation to the synthesis of WSX receptor or OB protein in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature* 293:620-625 (1981); Mantei *et al.*, *Nature* 281:40-46 (1979); EP 117,060; and EP 117,058. A

particularly useful plasmid for mammalian cell culture expression of WSX receptor or OB protein is pRK5 (EP 307,247) or pSVI6B. WO 91/08291 published 13 June 1991.

3. Selection and Transformation of Host Cells

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Strain W3110 is a particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins, with examples of such hosts including *E. coli* W3110 strain 27C7. The complete genotype of 27C7 is *tonAΔ ptr3 phoAΔE15 Δ(argF-lac)169 ompTΔ degP41kan*^{r}. Strain 27C7 was deposited on 30 October 1991 in the American Type Culture Collection as ATCC No. 55,244. Alternatively, the strain of *E. coli* having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990 may be employed. Alternatively still, methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for WSX receptor or OB protein-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* (Beach *et al., Nature,* 290:140 (1981); EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, *supra*) such as, *e.g.*, *K. lactis* (MW98-8C, CBS683, CBS4574), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906; Van den Berg *et al.*, *supra*), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, *J. Basic Microbiol.* 28:265-278 (1988)); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA* 76:5259-5263 (1979)); *Schwanniomyces* such as *Schwanniomyces*

occidentalis (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al., Biochem. Biophys. Res. Commun.* 112:284-289 (1983); Tilburn *et al., Gene* 26:205-221 (1983); Yelton *et al., Proc. Natl. Acad. Sci. USA* 81:1470-1474 (1984)) and *A. niger.* Kelly *et al., EMBO J.* 4:475-479 (1985).

Suitable host cells for the expression of glycosylated WSX receptor or OB protein are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. See, *e.g.*, Luckow *et al., Bio/Technology* 6:47-55 (1988); Miller *et al.*, in *Genetic Engineering*, Setlow *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al., Nature* 315:592-594 (1985). A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the WSX receptor or OB protein-encoding DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding the WSX receptor or OB protein is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the WSX receptor or OB protein-encoding DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al., J. Mol. Appl. Gen.* 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. See, *e.g., Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al., J. Gen Virol.* 36:59

(1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al., Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al., Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors for WSX receptor or OB protein production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene* 23:315 (1983) and WO 89/05859 published 29 June 1989. In addition, plants may be transfected using ultrasound treatment as described in WO 91/00358 published 10 January 1991.

For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham *et al., Virology* 52:456-457 (1978) is preferred. General aspects of mammalian cell host system transformations have been described in U.S. Pat. No. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al., J. Bact.* 130:946 (1977) and Hsiao *et al., Proc. Natl. Acad. Sci. USA* 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, *etc.*, may also be

used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology* 185:527-537 (1990) and Mansour *et al.*, *Nature* 336:348-352 (1988).

4. Culturing the Host Cells

Prokaryotic cells used to produce the WSX receptor or OB protein of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the WSX receptor or OB protein of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al. Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.*102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found in *Mammalian Cell Biotechnology: a Practical Approach*, M. Butler, ed. (IRL Press, 1991).

The host cells referred to in this disclosure encompass cells in culture as well as cells that are within a host animal.

5. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA* 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a

polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, *Am. J. Clin. Path.* 75:734-738 (1980).

Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared as described herein.

6. Purification of WSX Receptor or OB Protein

WSX receptor *(e.g.*, WSX receptor ECD) or OB protein preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates. If the WSX receptor is membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100)

When WSX receptor or OB protein is produced in a recombinant cell other than one of human origin, the WSX receptor or OB protein is completely free of proteins or polypeptides of human origin. However, it is necessary to purify WSX receptor or OB protein from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to WSX receptor or OB protein. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. WSX receptor or OB protein thereafter is purified from contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75™; and protein A Sepharose™ columns to remove contaminants such as IgG.

WSX receptor or OB protein variants in which residues have been deleted, inserted, or substituted are recovered in the same fashion as native sequence WSX receptor or OB protein, taking account of any substantial changes in properties occasioned by the variation. Immunoaffinity columns such as a rabbit polyclonal anti-WSX receptor or OB protein column can be employed to absorb the WSX receptor or OB protein variant by binding it to at least one remaining immune epitope.

A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants.

7. Covalent Modifications

Covalent modifications of WSX receptor or OB protein are included within the scope of this invention. Both native sequence WSX receptor or OB protein and amino acid sequence variants of the WSX receptor or OB protein may be covalently modified. One type of covalent modification of the WSX receptor or OB protein is introduced into the molecule by reacting targeted amino acid residues of the WSX receptor or OB protein with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the WSX receptor or OB protein.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing α-amino-containing residues include imidoesters such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of

arginine residues requires that the reaction be performed under alkaline conditions because of the high pK_a of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as with the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking WSX receptor or OB protein to a water-insoluble support matrix or surface for use in the method for purifying anti-WSX receptor or OB protein antibodies, and vice-versa. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-((p-azidophenyl)dithio)propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. These residues are deamidated under neutral or basic conditions. The deamidated form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the WSX receptor or OB protein included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in native WSX receptor or OB protein, and/or adding one or more glycosylation sites that are not present in the native WSX receptor or OB protein.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxylamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the WSX receptor or OB protein is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native WSX receptor or OB protein sequence (for O-linked glycosylation sites). For ease, the WSX receptor or OB protein amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the WSX receptor or OB protein at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above and in U.S. Pat. No. 5,364,934, *supra*.

Another means of increasing the number of carbohydrate moieties on the WSX receptor or OB protein is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin *et al., CRC Crit. Rev. Biochem.* 259-306 (1981).

Removal of carbohydrate moieties present on the WSX receptor or OB protein may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This

treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.* 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.* 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.* 138:350 (1987).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.*, *J. Biol. Chem.* 257:3105 (1982). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of WSX receptor or OB protein comprises linking the WSX receptor or OB protein to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

Since it is often difficult to predict in advance the characteristics of a variant WSX receptor or OB protein, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. A change in the immunological character of the WSX receptor or OB protein molecule, such as affinity for a given antibody, is also able to be measured by a competitive-type immunoassay. The WSX receptor variant is assayed for changes in the ability of the protein to induce cell proliferation in the colony assay of Example 8. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

8. Epitope-Tagged WSX Receptor or OB Protein

This invention encompasses chimeric polypeptides comprising WSX receptor or OB protein fused to a heterologous polypeptide. A chimeric WSX receptor or OB protein is one type of WSX receptor or OB protein variant as defined herein. In one preferred embodiment, the chimeric polypeptide comprises a fusion of the WSX receptor or OB protein with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally provided at the amino- or carboxyl- terminus of the WSX receptor or OB protein. Such epitope-tagged forms of the WSX receptor or OB protein are desirable as the presence thereof can be detected using a labeled antibody against the tag polypeptide. Also, provision of the epitope tag enables the WSX receptor or OB protein to be readily purified by affinity purification using the anti-tag antibody. Affinity purification techniques and diagnostic assays involving antibodies are described later herein.

Tag polypeptides and their respective antibodies are well known in the art. Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al.*, *Mol. Cell. Biol.* 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al.*, *Molecular and Cellular Biology* 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody. Paborsky *et al.*, *Protein Engineering* 3(6):547-553 (1990). Other tag polypeptides have been disclosed. Examples include the Flag-peptide (Hopp *et al.*, *BioTechnology* 6:1204-1210 (1988)); the KT3 epitope peptide (Martin *et al.*, *Science* 255:192-194 (1992)); an α-tubulin epitope peptide (Skinner *et al.*, *J. Biol. Chem.* 266:15163-15166 (1991)); and the T7 gene 10 protein peptide tag. Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA* 87:6393-6397 (1990). Once the tag polypeptide has been selected, an antibody thereto can be generated using the techniques disclosed herein.

The general methods suitable for the construction and production of epitope-tagged WSX receptor or OB protein are the same as those disclosed hereinabove. WSX receptor or OB protein-tag polypeptide fusions are most conveniently constructed by fusing the cDNA sequence encoding the WSX receptor or OB protein portion in-frame to the tag polypeptide DNA sequence and expressing the resultant DNA fusion construct in appropriate host cells. Ordinarily, when preparing the WSX receptor or OB protein-tag polypeptide chimeras of the present invention, nucleic acid encoding the WSX receptor or OB protein will be fused at its 3' end to nucleic acid encoding the N-terminus of the tag polypeptide, however 5' fusions are also possible.

Epitope-tagged WSX receptor or OB protein can be conveniently purified by affinity chromatography using the anti-tag antibody. The matrix to which the affinity antibody is attached is most often agarose, but other matrices are available (*e.g.* controlled pore glass or poly(styrenedivinyl)benzene). The epitope-tagged WSX receptor or OB protein can be eluted from the affinity column by varying the buffer pH or ionic strength or adding chaotropic agents, for example.

9. WSX Receptor or OB Protein Immunoadhesins

Chimeras constructed from a receptor sequence linked to an appropriate immunoglobulin constant domain sequence (immunoadhesins) are known in the art. Immunoadhesins reported in the literature include fusions of the T cell receptor* (Gascoigne *et al.*, *Proc. Natl.Acad. Sci. USA* 84: 2936-2940 (1987)); CD4* (Capon *et al.*, *Nature* 337: 525-531 (1989); Traunecker *et al.*, *Nature* 339: 68-70 (1989); Zettmeissl *et al.*, *DNA Cell Biol. USA* 9: 347-353 (1990); Byrn *et al.*, *Nature* 344: 667-670 (1990)); L-selectin (homing receptor) ((Watson *et al.*, *J. Cell. Biol.* 110:2221-2229 (1990); Watson *et al.*, *Nature* 349: 164-167 (1991)); CD44* (Aruffo *et al.*, *Cell* 61: 1303-1313 (1990)); CD28*

and B7* (Linsley *et al.*, *J. Exp. Med.* 173: 721-730 (1991)); CTLA-4* (Lisley *et al.*, *J. Exp. Med.* 174: 561-569 (1991)); CD22* (Stamenkovic *et al.*, *Cell* 66:1133-1144 (1991)); TNF receptor (Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 10535-10539 (1991); Lesslauer *et al.*, *Eur. J. Immunol.* 27: 2883-2886 (1991); Peppel *et al.*, *J. Exp. Med.* 174:1483-1489 (1991)); NP receptors (Bennett *et al.*, *J. Biol. Chem.* 266:23060-23067 (1991)); and IgE receptor α^* (Ridgway *et al.*, *J. Cell. Biol.* 115:abstr. 1448 (1991)), where the asterisk (*) indicates that the receptor is member of the immunoglobulin superfamily.

The simplest and most straightforward immunoadhesin design combines the binding region(s) of the "adhesin" protein with the hinge and Fc regions of an immunoglobulin heavy chain. Ordinarily, when preparing the WSX receptor or OB-immunoglobulin chimeras of the present invention, nucleic acid encoding OB protein or the extracellular domain of the WSX receptor will be fused C-terminally to nucleic acid encoding the N-terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible. For OB-immunoglobulin chimeras, an OB protein fragment which retains the ability to bind to the WSX receptor may be employed.

Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally active hinge, CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the CH1 of the heavy chain or the corresponding region of the light chain.

The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion or binding characteristics of the WSX receptor or OB-immunoglobulin chimeras.

In some embodiments, the WSX receptor or OB-immunoglobulin chimeras are assembled as monomers, or hetero- or homo-multimers, and particularly as dimers or tetramers, essentially as illustrated in WO 91/08298.

In a preferred embodiment, the OB protein sequence or WSX receptor extracellular domain sequence is fused to the N-terminus of the C-terminal portion of an antibody (in particular the Fc domain), containing the effector functions of an immunoglobulin, *e.g.* immunoglobulin G1 (IgG1). It is possible to fuse the entire heavy chain constant region to the OB protein or WSX receptor extracellular domain sequence. However, more preferably, a sequence beginning in the hinge region just upstream of the papain cleavage site (which defines IgG Fc chemically; residue 216, taking the first residue of heavy chain constant region to be 114, or analogous sites of other immunoglobulins) is used in the fusion. In a particularly preferred embodiment, the OB protein or WSX receptor amino acid sequence is fused to the hinge region, CH2 and CH3, or the CH1, hinge, CH2 and CH3

domains of an IgG1, IgG2, or IgG3 heavy chain. The precise site at which the fusion is made is not critical, and the optimal site can be determined by routine experimentation.

In some embodiments, the WSX receptor or OB-immunoglobulin chimeras are assembled as multimers, and particularly as homo-dimers or -tetramers. Generally, these assembled immunoglobulins will have known unit structures. A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of basic four units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in multimeric form in serum. In the case of multimer, each four unit may be the same or different.

Various exemplary assembled WSX receptor or OB-immunoglobulin chimeras within the scope herein are schematically diagrammed below:

(a) AC_L-AC_L;

(b) AC_H-(AC_H, AC_L-AC_H, AC_L-V_HC_H, or V_LC_L-AC_H);

(c) AC_L-AC_H-(AC_L-AC_H, AC_L-V_HC_H, V_LC_L-AC_H, or V_LC_L-V_HC_H);

(d) AC_L-V_HC_H-(AC_H, or AC_L-V_HC_H, or V_LC_L-AC_H);

(e) V_LC_L-AC_H-(AC_L-V_HC_H, or V_LC_L-AC_H); and

(f) $(A\text{-}Y)_n\text{-}(V_LC_L\text{-}V_HC_H)_2$,

wherein

each A represents identical or different OB protein or WSX receptor amino acid sequences;

V_L is an immunoglobulin light chain variable domain;

V_H is an immunoglobulin heavy chain variable domain;

C_L is an immunoglobulin light chain constant domain;

C_H is an immunoglobulin heavy chain constant domain;

n is an integer greater than 1;

Y designates the residue of a covalent cross-linking agent.

In the interests of brevity, the foregoing structures only show key features; they do not indicate joining (J) or other domains of the immunoglobulins, nor are disulfide bonds shown. However, where such domains are required for binding activity, they shall be constructed as being present in the ordinary locations which they occupy in the immunoglobulin molecules.

Alternatively, the OB protein or WSX receptor extracellular domain sequence can be inserted between immunoglobulin heavy chain and light chain sequences such that an immunoglobulin comprising a chimeric heavy chain is obtained. In this embodiment, the OB protein or WSX receptor sequence is fused to the 3' end of an immunoglobulin heavy chain in each arm of an immunoglobulin, either between the hinge and the CH2 domain, or between the CH2 and CH3

domains. Similar constructs have been reported by Hoogenboom *et al.*, *Mol. Immunol.*, 28:1027-1037 (1991).

Although the presence of an immunoglobulin light chain is not required in the immunoadhesins of the present invention, an immunoglobulin light chain might be present either covalently associated to an OB protein or WSX receptor-immunoglobulin heavy chain fusion polypeptide, or directly fused to the WSX receptor extracellular domain or OB protein. In the former case, DNA encoding an immunoglobulin light chain is typically coexpressed with the DNA encoding the OB protein or WSX receptor-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain and the light chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chain-light chain pairs. Methods suitable for the preparation of such structures are, for example, disclosed in U.S. Patent No. 4,816,567 issued 28 March 1989.

In a preferred embodiment, the immunoglobulin sequences used in the construction of the immunoadhesins of the present invention are from an IgG immunoglobulin heavy chain constant domain. For human immunoadhesins, the use of human IgG1 and IgG3 immunoglobulin sequences is preferred. A major advantage of using IgG1 is that IgG1 immunoadhesins can be purified efficiently on immobilized protein A. In contrast, purification of IgG3 requires protein G, a significantly less versatile medium. However, other structural and functional properties of immunoglobulins should be considered when choosing the Ig fusion partner for a particular immunoadhesin construction. For example, the IgG3 hinge is longer and more flexible, so it can accommodate larger adhesin domains that may not fold or function properly when fused to IgG1. Another consideration may be valency; IgG immunoadhesins are bivalent homodimers, whereas Ig subtypes like IgA and IgM may give rise to dimeric or pentameric structures, respectively, of the basic Ig homodimer unit. For immunoadhesins designed for *in vivo* application, the pharmacokinetic properties and the effector functions specified by the Fc region are important as well. Although IgG1, IgG2 and IgG4 all have *in vivo* half-lives of 21 days, their relative potencies at activating the complement system are different. IgG4 does not activate complement, and IgG2 is significantly weaker at complement activation than IgG1. Moreover, unlike IgG1, IgG2 does not bind to Fc receptors on mononuclear cells or neutrophils. While IgG3 is optimal for complement activation, its *in vivo* half-life is approximately one third of the other IgG isotypes. Another important consideration for immunoadhesins designed to be used as human therapeutics is the number of allotypic variants of the particular isotype. In general, IgG isotypes with fewer serologically-defined allotypes are preferred. For example, IgG1 has only four serologically-defined allotypic sites, two of which (G1m and 2) are located in the Fc region; and one of these sites G1m1, is non-immunogenic. In contrast,

there are 12 serologically-defined allotypes in IgG3, all of which are in the Fc region; only three of these sites (G3m5, 11 and 21) have one allotype which is nonimmunogenic. Thus, the potential immunogenicity of a γ3 immunoadhesin is greater than that of a γ1 immunoadhesin.

With respect to the parental immunoglobulin, a useful joining point is just upstream of the cysteines of the hinge that form the disulfide bonds between the two heavy chains. In a frequently used design, the codon for the C-terminal residue of the WSX receptor or OB protein part of the molecule is placed directly upstream of the codons for the sequence DKTHTCPPCP (SEQ ID NO:44) of the IgG1 hinge region.

The general methods suitable for the construction and expression of immunoadhesins are the same as those disclosed hereinabove with regard to WSX receptor and OB protein. Immunoadhesins are most conveniently constructed by fusing the cDNA sequence encoding the WSX receptor or OB protein portion in-frame to an Ig cDNA sequence. However, fusion to genomic Ig fragments can also be used (see, *e.g.,* Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:2936-2940 (1987); Aruffo *et al.*, *Cell* 61:1303-1313 (1990); Stamenkovic *et al.*, *Cell* 66:1133-1144 (1991)). The latter type of fusion requires the presence of Ig regulatory sequences for expression. cDNAs encoding IgG heavy-chain constant regions can be isolated based on published sequence from cDNA libraries derived from spleen or peripheral blood lymphocytes, by hybridization or by polymerase chain reaction (PCR) techniques. The cDNAs encoding the WSX receptor or OB protein and Ig parts of the immunoadhesin are inserted in tandem into a plasmid vector that directs efficient expression in the chosen host cells. For expression in mammalian cells, pRK5-based vectors (Schall *et al.*, *Cell* 61:361-370 (1990)) and CDM8-based vectors (Seed, *Nature* 329:840 (1989)) can be used. The exact junction can be created by removing the extra sequences between the designed junction codons using oligonucleotide-directed deletional mutagenesis (Zoller *et al.*, *Nucleic Acids Res.* 10:6487 (1982); Capon *et al.*, *Nature* 337:525-531 (1989)). Synthetic oligonucleotides can be used, in which each half is complementary to the sequence on either side of the desired junction; ideally, these are 36 to 48-mers. Alternatively, PCR techniques can be used to join the two parts of the molecule in-frame with an appropriate vector.

The choice of host cell line for the expression of the immunoadhesin depends mainly on the expression vector. Another consideration is the amount of protein that is required. Milligram quantities often can be produced by transient transfections. For example, the adenovirus EIA-transformed 293 human embryonic kidney cell line can be transfected transiently with pRK5-based vectors by a modification of the calcium phosphate method to allow efficient immunoadhesin expression. CDM8-based vectors can be used to transfect COS cells by the DEAE-dextran method (Aruffo *et al.*, *Cell* 61:1303-1313 (1990); Zettmeissl *et al.*, *DNA Cell Biol. US* 9:347-353 (1990)). If

larger amounts of protein are desired, the immunoadhesin can be expressed after stable transfection of a host cell line. For example, a pRK5-based vector can be introduced into Chinese hamster ovary (CHO) cells in the presence of an additional plasmid encoding dihydrofolate reductase (DHFR) and conferring resistance to G418. Clones resistant to G418 can be selected in culture; these clones are grown in the presence of increasing levels of DHFR inhibitor methotrexate; clones are selected, in which the number of gene copies encoding the DHFR and immunoadhesin sequences is co-amplified. If the immunoadhesin contains a hydrophobic leader sequence at its N-terminus, it is likely to be processed and secreted by the transfected cells. The expression of immunoadhesins with more complex structures may require uniquely suited host cells; for example, components such as light chain or J chain may be provided by certain myeloma or hybridoma cell hosts (Gascoigne *et al.*, 1987, *supra,* Martin *et al.*, *J. Virol.* 67:3561-3568 (1993)).

Immunoadhesins can be conveniently purified by affinity chromatography. The suitability of protein A as an affinity ligand depends on the species and isotype of the immunoglobulin Fc domain that is used in the chimera. Protein A can be used to purify immunoadhesins that are based on human γ1, γ2, or γ4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. The conditions for binding an immunoadhesin to the protein A or G affinity column are dictated entirely by the characteristics of the Fc domain; that is, its species and isotype. Generally, when the proper ligand is chosen, efficient binding occurs directly from unconditioned culture fluid. One distinguishing feature of immunoadhesins is that, for human γ1 molecules, the binding capacity for protein A is somewhat diminished relative to an antibody of the same Fc type. Bound immunoadhesin can be efficiently eluted either at acidic pH (at or above 3.0), or in a neutral pH buffer containing a mildly chaotropic salt. This affinity chromatography step can result in an immunoadhesin preparation that is >95% pure.

Other methods known in the art can be used in place of, or in addition to, affinity chromatography on protein A or G to purify immunoadhesins. Immunoadhesins behave similarly to antibodies in thiophilic gel chromatography (Hutchens *et al., Anal. Biochem.* 159:217-226 (1986)) and immobilized metal chelate chromatography (Al-Mashikhi *et al., J. Dairy Sci.* 71:1756-1763 (1988)). In contrast to antibodies, however, their behavior on ion exchange columns is dictated not only by their isoelectric points, but also by a charge dipole that may exist in the molecules due to their chimeric nature.

If desired, the immunoadhesins can be made bispecific. Thus, the immunoadhesins of the present invention may combine a WSX receptor extracellular domain and a domain, such as the extracellular domain, of another cytokine receptor subunit. Exemplary cytokine receptors from which such bispecific immunoadhesin molecules can be made include TPO (or *mpl* ligand), EPO, G-CSF, IL-4, IL-7, GH, PRL, IL-3, GM-CSF, IL-5, IL-6, LIF, OSM,CNTF and IL-2 receptors. Alternatively, an OB protein domain may be combined with another cytokine, such as those exemplified herein, in the generation of a bispecific immunoadhesin. For bispecific molecules, trimeric molecules, composed of a chimeric antibody heavy chain in one arm and a chimeric antibody heavy chain-light chain pair in the other arm of their antibody-like structure are advantageous, due to ease of purification. In contrast to antibody-producing quadromas traditionally used for the production of bispecific immunoadhesins, which produce a mixture of ten tetramers, cells transfected with nucleic acid encoding the three chains of a trimeric immunoadhesin structure produce a mixture of only three molecules, and purification of the desired product from this mixture is correspondingly easier.

10. Long Half-Life Derivatives of OB Protein

Prefered OB protein functional derivatives for use in the methods of the present invention include OB-immunoglobulin chimeras (immunoadhesins) and other longer half-life molecules. Techniques for generating OB protein immunoadhesins have been described above. The prefered OB immunoadhesin is made according to the techniques described in Example 11 below.

Other derivatives of the OB proteins, which possess a longer half-life than the native molecules comprise the OB protein or an OB-immunoglobulin chimera covalently bonded to a nonproteinaceous polymer. The nonproteinaceous polymer ordinarily is a hydrophilic synthetic polymer, *i.e.*, a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or *in vitro* methods are useful, as are polymers which are isolated from native sources. Hydrophilic polyvinyl polymers fall within the scope of this invention, *e.g.* polyvinylalcohol and polyvinylpyrrolidone. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG); polyelkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronics™); polymethacrylates; carbomers; branched or unbranched polysaccharides which comprise the saccharide monomers D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, D-glucuronic acid, sialic acid, D-galacturonic acid, D-mannuronic acid (*e.g.* polymannuronic acid, or alginic acid), D-glucosamine, D-galactosamine, D-glucose and neuraminic acid including homopolysaccharides and heteropolysaccharides such as lactose, amylopectin, starch, hydroxyethyl starch, amylose, dextrane sulfate, dextran, dextrins, glycogen, or the polysaccharide subunit of acid mucopolysaccharides, *e.g.* hyaluronic acid; polymers of sugar alcohols such as polysorbitol and polymannitol; heparin or

heparon. The polymer prior to cross-linking need not be, but preferably is, water soluble, but the final conjugate must be water soluble. In addition, the polymer should not be highly immunogenic in the conjugate form, nor should it possess viscosity that is incompatible with intravenous infusion or injection if it is intended to be administered by such routes.

Preferably the polymer contains only a single group which is reactive. This helps to avoid cross-linking of protein molecules. However, it is within the scope herein to optimize reaction conditions to reduce cross-linking, or to purify the reaction products through gel filtration or chromatographic sieves to recover substantially homogenous derivatives.

The molecular weight of the polymer may desirably range from about 100 to 500,000, and preferably is from about 1,000 to 20,000. The molecular weight chosen will depend upon the nature of the polymer and the degree of substitution. In general, the greater the hydrophilicity of the polymer and the greater the degree of substitution, the lower the molecular weight that can be employed. Optimal molecular weights will be determined by routine experimentation.

The polymer generally is covalently linked to the OB protein or to the OB-immunoglobulin chimera though a multifunctional crosslinking agent which reacts with the polymer and one or more amino acid or sugar residues of the OB protein or OB-immunoglobulin chimera to be linked. However, it is within the scope of the invention to directly crosslink the polymer by reacting a derivatized polymer with the hybrid, or via versa.

The covalent crosslinking site on the OB protein or OB-immunoglobulin chimera includes the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the hybrid without the use of a multifunctional (ordinarily bifunctional) crosslinking agent. Covalent binding to amino groups is accomplished by known chemistries based upon cyanuric chloride, carbonyl diimidazole, aldehyde reactive groups (PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, or PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, succinimidyl active esters, activated dithiocarbonate PEG, 2,4,5-trichlorophenylcloroformate or P-nitrophenylcloroformate activated PEG). Carboxyl groups are derivatized by coupling PEG-amine using carbodiimide.

Polymers are conjugated to oligosaccharide groups by oxidation using chemicals, *e.g.* metaperiodate, or enzymes, *e.g.* glucose or galactose oxidase (either of which produces the aldehyde derivative of the carbohydrate), followed by reaction with hydrazide or amino derivatized polymers, in the same fashion as is described by Heitzmann *et al.*, *P.N.A.S.* 71:3537-41 (1974) or Bayer *et al.*, *Methods in Enzymology* 62:310 (1979), for the labeling of oligosaccharides with biotin or avidin. Further, other chemical or enzymatic methods which have been used heretofore to link

oligosaccharides are particularly advantageous because, in general, there are fewer substitutions than amino acid sites for derivatization, and the oligosaccharide products thus will be more homogenous. The oligosaccharide substituents also are optionally modified by enzyme digestion to remove sugars, *e.g.* by neuraminidase digestion, prior to polymer derivatization.

The polymer will bear a group which is directly reactive with an amino acid side chain, or the N- or C-terminus of the polypeptide linked, or which is reactive with the multifunctional cross-linking agent. In general, polymers bearing such reactive groups are known for the preparation of immobilized proteins. In order to use such chemistries here, one should employ a water soluble polymer otherwise derivatized in the same fashion as insoluble polymers heretofore employed for protein immobilization. Cyanogen bromide activation is a particularly useful procedure to employ in crosslinking polysaccharides.

"Water soluble" in reference to the starting polymer means that the polymer or its reactive intermediate used for conjugation is sufficiently water soluble to participate in a derivatization reaction.

"Water soluble" in reference to the polymer conjugate means that the conjugate is soluble in physiological fluids such as blood.

The degree of substitution with such a polymer will vary depending upon the number of reactive sites on the protein, whether all or a fragment of the protein is used, whether the protein is a fusion with a heterologous protein (*e.g.* an OB-immunoglobulin chimera), the molecular weight, hydrophilicity and other characteristics of the polymer, and the particular protein derivatization sites chosen. In general, the conjugate contains about from 1 to 10 polymer molecules, while any heterologous sequence may be substituted with an essentially unlimited number of polymer molecules so long as the desired activity is not significantly adversely affected. The optimal degree of cross-linking is easily determined by an experimental matrix in which the time, temperature and other reaction conditions are varied to change the degree of substitution, after which the ability of the conjugates to function in the desired fashion is determined.

The polymer, *e.g.* PEG, is cross-linked by a wide variety of methods known *per se* for the covalent modification of proteins with nonproteinaceous polymers such as PEG. Certain of these methods, however, are not preferred for the purposes herein. Cyanuronic chloride chemistry leads to many side reactions, including protein cross-linking. In addition, it may be particularly likely to lead to inactivation of proteins containing sulfhydryl groups. Carbonyl diimidazole chemistry (Beauchamp *et al.*, *Anal Biochem.* 131:25-33 (1983)) requires high pH (>8.5), which can inactivate proteins. Moreover, since the "activated PEG" intermediate can react with water, a very large molar excess of "activated PEG" over protein is required. The high concentrations of PEG required for the

carbonyl diimidazole chemistry also led to problems in purification, as both gel filtration chromatography and hydrophilic interaction chromatography are adversely affected. In addition, the high concentrations of "activated PEG" may precipitate protein, a problem that *per se* has been noted previously (Davis, U.S. Patent No. 4,179,337). On the other hand, aldehyde chemistry (Royer, U.S. Patent No. 4,002,531) is more efficient since it requires only a 40-fold molar excess of PEG and a 1-2 hr incubation. However, the manganese dioxide suggested by Royer for preparation of the PEG aldehyde is problematic "because of the pronounced tendency of PEG to form complexes with metal-based oxidizing agents" (Harris *et al.*, *J. Polym. Sci. Polym. Chem. Ed.* 22:341-52 (1984)). The use of a Moffatt oxidation, utilizing DMSO and acetic anhydride, obviates this problem. In addition, the sodium borohydride suggested by Royer must be used at high pH and has a significant tendency to reduce disulfide bonds. In contrast, sodium cyanoborohydride, which is effective at neutral pH and has very little tendency to reduce disulfide bonds is preferred.

Functionalized PEG polymers to modify the OB protein or OB-immunoglobulin chimeras of the present invention are available from Shearwater Polymers, Inc. (Huntsville, AL). Such commercially available PEG derivatives include, but are not limited to, amino-PEG, PEG amino acid esters, PEG-hydrazide, PEG-thiol, PEG-succinate, carboxymethylated PEG, PEG-propionic acid, PEG amino acids, PEG succinimidyl succinate, PEG succinimidyl propionate, succinimidyl ester of carboxymethylated PEG, succinimidyl carbonate of PEG, succinimidyl esters of amino acid PEGs, PEG-oxycarbonylimidazole, PEG-nitrophenyl carbonate, PEG tresylate, PEG-glycidyl ether, PEG-aldehyde, PEG vinylsulfone, PEG-maleimide, PEG-orthopyridyl-disulfide, heterofunctional PEGs, PEG vinyl derivatives, PEG silanes, and PEG phospholides. The reaction conditions for coupling these PEG derivatives will vary depending on the protein, the desired degree of PEGylation, and the PEG derivative utilized. Some factors involved in the choice of PEG derivatives include: the desired point of attachment (lysine or cysteine), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

The long half-life conjugates of this invention are separated from the unreacted starting materials by gel filtration. Heterologous species of the conjugates are purified from one another in the same fashion. The polymer also may be water-insoluble, as a hydrophilic gel.

The conjugates may also be purified by ion-exchange chromatography. The chemistry of many of the electrophilically activated PEG's results in a reduction of amino group charge of the PEGylated product. Thus, high resolution ion exchange chromatography can be used to separate the free and conjugated proteins, and to resolve species with different levels of PEGylation. In fact,

the resolution of different species (*e.g.* containing one or two PEG residues) is also possible due to the difference in the ionic properties of the unreacted amino acids.

B. Therapeutic Uses for the WSX Receptor

The WSX receptor and WSX receptor gene are believed to find therapeutic use for administration to a mammal in the treatment of diseases characterized by a decrease in hematopoietic cells. Examples of these diseases include: anemia (including macrocytic and aplastic anemia); thrombocytopenia; hypoplasia; disseminated intravascular coagulation (DIC); myelodysplasia; immune (autoimmune) thrombocytopenic purpura (ITP); and HIV induced ITP. Additionally, these WSX receptor molecules may be useful in treating myeloproliferative thrombocytotic diseases as well as thrombocytosis from inflammatory conditions and in iron deficiency. WSX receptor polypeptide and WSX receptor gene which lead to an increase in hematopoietic cell proliferation may also be used to enhance repopulation of mature blood cell lineages in cells having undergone chemo- or radiation therapy or bone marrow transplantation therapy. Generally, the WSX receptor molecules are expected to lead to an enhancement of the proliferation and/or differentiation (but especially proliferation) of primitive hematopoietic cells.

Other potential therapeutic applications for WSX receptor and WSX receptor gene include the treatment of obesity and diabetes and for promoting kidney, liver and lung growth and/or repair (*e.g.* in renal failure). WSX receptor can also be used to treat obesity-related conditions, such as type II adult onset diabetes, infertility, hypercholesterolemia, hyperlipidemia, cardiovascular disease and hypertension.

The WSX receptor may be administered alone or in combination with cytokines (such as OB protein), growth factors or antibodies in the above-identified clinical situations. This may facilitate an effective lowering of the dose of WSX receptor. Suitable dosages for such additional molecules will be discussed below.

Administration of WSX receptor to a mammal having depressed levels of endogenous WSX receptor or a defective WSX receptor gene is contemplated, preferably in the situation where such depressed levels lead to a pathological disorder, or where there is lack of activation of the WSX receptor. In these embodiments where the full length WSX receptor is to be administered to the patient, it is contemplated that the gene encoding the receptor may be administered to the patient via gene therapy technology.

In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time

or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:4143-4146 (1986)). The oligonucleotides can be modified to enhance their uptake, *e.g.*, by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, *Trends in Biotechnology* 11:205-210 (1993)). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992).

The invention also provides antagonists of WSX receptor activation (*e.g.* WSX receptor ECD, WSX receptor immunoadhesins and WSX receptor antisense nucleic acid; neutralizing antibodies and uses thereof are discussed in section E below). Administration of WSX receptor antagonist to a mammal having increased or excessive levels of endogenous WSX receptor activation is contemplated, preferably in the situation where such levels of WSX receptor activation lead to a pathological disorder.

In one embodiment, WSX receptor antagonist molecules may be used to bind endogenous ligand in the body, thereby causing desensitized WSX receptors to become responsive to WSX ligand, especially when the levels of WSX ligand in the serum exceed normal physiological levels.

Also, it may be beneficial to bind endogenous WSX ligand which is activating undesired cellular responses (such as proliferation of tumor cells). Potential therapeutic applications for WSX antagonists include for example, treatment of metabolic disorders (*e.g.,* anorexia, cachexia, steroid-induced truncalobesity and other wasting diseases characterized by loss of appetite, diminished food intake or body weight loss), stem cell tumors and other tumors which express WSX receptor.

Pharmaceutical compositions of the WSX receptor ECD may further include a WSX ligand. Such dual compositions may be beneficial where it is therapeutically useful to prolong half-life of WSX ligand, and/or activate endogenous WSX receptor directly as a heterotrimeric complex.

Therapeutic formulations of WSX receptor are prepared for storage by mixing WSX receptor having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences*, 16th edition, Osol, A., Ed., (1980)), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as Tween, Pluronics™ or polyethylene glycol (PEG).

The WSX receptor also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, *supra*.

WSX receptor to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. WSX receptor ordinarily will be stored in lyophilized form or in solution.

Therapeutic WSX receptor compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of WSX receptor administration is in accord with known methods, *e.g.*, those routes set forth above for specific indications, as well as the general routes of injection or infusion

by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional means, or sustained release systems as noted below. WSX receptor is administered continuously by infusion or by bolus injection. Generally, where the disorder permits, one should formulate and dose the WSX receptor for site-specific delivery.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (*e.g.*, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *J. Biomed. Mater. Res.* 15:167-277 (1981) and Langer, *Chem. Tech.* 12:98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and γ ethyl-L-glutamate (Sidman *et al.*, *Biopolymers* 22:547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release WSX receptor compositions also include liposomally entrapped WSX receptor. Liposomes containing WSX receptor are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal WSX receptor therapy.

When applied topically, the WSX receptor is suitably combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except

that they must be physiologically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The compositions also may be impregnated into transdermal patches, plasters, and bandages, preferably in liquid or semi-liquid form.

For obtaining a gel formulation, the WSX receptor formulated in a liquid composition may be mixed with an effective amount of a water-soluble polysaccharide or synthetic polymer such as PEG to form a gel of the proper viscosity to be applied topically. The polysaccharide that may be used includes, for example, cellulose derivatives such as etherified cellulose derivatives, including alkyl celluloses, hydroxyalkyl celluloses, and alkylhydroxyalkyl celluloses, for example, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; starch and fractionated starch; agar; alginic acid and alginates; gum arabic; pullullan; agarose; carrageenan; dextrans; dextrins; fructans; inulin; mannans; xylans; arabinans; chitosans; glycogens; glucans; and synthetic biopolymers; as well as gums such as xanthan gum; guar gum; locust bean gum; gum arabic; tragacanth gum; and karaya gum; and derivatives and mixtures thereof. The preferred gelling agent herein is one that is inert to biological systems, nontoxic, simple to prepare, and not too runny or viscous, and will not destabilize the WSX receptor held within it.

Preferably the polysaccharide is an etherified cellulose derivative, more preferably one that is well defined, purified, and listed in USP, *e.g.*, methylcellulose and the hydroxyalkyl cellulose derivatives, such as hydroxypropyl cellulose, hydroxyethyl cellulose, and hydroxypropyl methylcellulose. Most preferred herein is methylcellulose.

The polyethylene glycol useful for gelling is typically a mixture of low and high molecular weight PEGs to obtain the proper viscosity. For example, a mixture of a PEG of molecular weight 400-600 with one of molecular weight 1500 would be effective for this purpose when mixed in the proper ratio to obtain a paste.

The term "water soluble" as applied to the polysaccharides and PEGs is meant to include colloidal solutions and dispersions. In general, the solubility of the cellulose derivatives is determined by the degree of substitution of ether groups, and the stabilizing derivatives useful herein should have a sufficient quantity of such ether groups per anhydroglucose unit in the cellulose chain to render the derivatives water soluble. A degree of ether substitution of at least 0.35 ether groups per anhydroglucose unit is generally sufficient. Additionally, the cellulose derivatives may be in the form of alkali metal salts, for example, the Li, Na, K, or Cs salts.

If methylcellulose is employed in the gel, preferably it comprises about 2-5%, more preferably about 3%, of the gel and the WSX receptor is present in an amount of about 300-1000 mg per ml of gel.

An effective amount of WSX receptor to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the WSX receptor until a dosage is reached that achieves the desired effect. A typical daily dosage for systemic treatment might range from about 1 μg/kg to up to 10 mg/kg or more, depending on the factors mentioned above. As an alternative general proposition, the WSX receptor is formulated and delivered to the target site or tissue at a dosage capable of establishing in the tissue a WSX receptor level greater than about 0.1 ng/cc up to a maximum dose that is efficacious but not unduly toxic. This intra-tissue concentration should be maintained if possible by continuous infusion, sustained release, topical application, or injection at empirically determined frequencies. The progress of this therapy is easily monitored by conventional assays.

C. Non-Therapeutic Uses for the WSX Receptor

WSX receptor nucleic acid is useful for the preparation of WSX receptor polypeptide by recombinant techniques exemplified herein which can then be used for production of anti-WSX receptor antibodies having various utilities described below.

The WSX receptor (polypeptide or nucleic acid) can be used to induce proliferation and/or differentiation of cells *in vitro*. In particular, it is contemplated that this molecule may be used to induce proliferation of stem cell/progenitor cell populations (*e.g.* CD34+ cell populations obtained as described in Example 8 below). These cells which are to be grown *ex vivo* may simultaneously be exposed to other known growth factors or cytokines, such as those described herein. This results in proliferation and/or differentiation of the cells having the WSX receptor.

In yet another aspect of the invention, the WSX receptor may be used for affinity purification of WSX ligand. Briefly, this technique involves: (a) contacting a source of WSX ligand with an immobilized WSX receptor under conditions whereby the WSX ligand to be purified is selectively adsorbed onto the immobilized receptor; (b) washing the immobilized WSX receptor and its support to remove non-adsorbed material; and (c) eluting the WSX ligand molecules from the immobilized WSX receptor to which they are adsorbed with an elution buffer. In a particularly preferred embodiment of affinity purification, WSX receptor is covalently attaching to an inert and porous matrix (*e.g.*, agarose reacted with cyanogen bromide). Especially preferred is a WSX receptor immunoadhesin immobilized on a protein A column. A solution containing WSX ligand is then passed

through the chromatographic material. The WSX ligand adsorbs to the column and is subsequently released by changing the elution conditions (*e.g.* by changing pH or ionic strength).

The WSX receptor may be used for competitive screening of potential agonists or antagonists for binding to the WSX receptor. Such agonists or antagonists may constitute potential therapeutics for treating conditions characterized by insufficient or excessive WSX receptor activation, respectively.

The preferred technique for identifying molecules which bind to the WSX receptor utilizes a chimeric receptor (*e.g.*, epitope tagged WSX receptor or WSX receptor immunoadhesin) attached to a solid phase, such as the well of an assay plate. Binding of molecules which are optionally labelled (*e.g.*, radiolabelled) to the immobilized receptor can be evaluated.

To identify WSX receptor agonists or antagonists, the thymidine incorporation assay can be used. For screening for antagonists, the WSX receptor can be exposed to a WSX ligand followed by the putative antagonist, or the WSX ligand and antagonist can be added to the WSX receptor simultaneously, and the ability of the antagonist to block receptor activation can be evaluated.

The WSX receptor polypeptides are also useful as molecular weight markers. To use a WSX receptor polypeptide as a molecular weight marker, gel filtration chromatography or SDS-PAGE, for example, will be used to separate protein(s) for which it is desired to determine their molecular weight(s) in substantially the normal way. The WSX receptor and other molecular weight markers will be used as standards to provide a range of molecular weights. For example, phosphorylase b (mw = 97,400), bovine serum albumin (mw = 68,000), ovalbumin (mw = 46,000), WSX receptor (mw = 44,800), trypsin inhibitor (mw = 20,100), and lysozyme (mw = 14,400) can be used as mw markers. The other molecular weight markers mentioned here can be purchased commercially from Amersham Corporation, Arlington Heights, IL. The molecular weight markers are generally labeled to facilitate detection thereof. For example, the markers may be biotinylated and following separation can be incubated with streptavidin-horseradish peroxidase so that the various markers can be detected by light detection.

The purified WSX receptor, and the nucleic acid encoding it, may also be sold as reagents for mechanism studies of WSX receptor and its ligands, to study the role of the WSX receptor and WSX ligand in normal growth and development, as well as abnormal growth and development, *e.g.,* in malignancies.

WSX receptor variants are useful as standards or controls in assays for the WSX receptor for example ELISA, RIA, or RRA, provided that they are recognized by the analytical system employed, *e.g.*, an anti-WSX receptor antibody.

D. WSX Receptor Antibody Preparation

1. Polyclonal antibodies

Polyclonal antibodies are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. In that the preferred epitope is in the ECD of the WSX receptor, it is desirable to use WSX receptor ECD or a molecule comprising the ECD (*e.g.*, WSX receptor immunoadhesin) as the antigen for generation of polyclonal and monoclonal antibodies. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining 1 mg or 1 µg of the peptide or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

2. Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (Cabilly *et al.*, *supra*).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes

may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al., Anal. Biochem.* 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.* 5:256-262 (1993) and Plückthun, *Immunol. Revs.* 130:151-188 (1992).

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature* 348:552-554 (1990). Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Mark *et al.*, *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (Cabilly *et al.*, *supra*; Morrison, *et al.*, *Proc. Nat. Acad. Sci. USA* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

3. Humanized and human antibodies

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly *et al.*, *supra*), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.* 151:2296 (1993); Chothia *et al., J. Mol. Biol.* 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); Presta *et al.*, *J. Immnol.* 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be

selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.* 7:33 (1993). Human antibodies can also be produced in phage- display libraries (Hoogenboom *et al.*, *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*222:581 (1991)).

4. Bispecific antibodies

Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different antigens. BsAbs can be used as tumor targeting or imaging agents and can be used to target enzymes or toxins to a cell possessing the WSX receptor. Such antibodies can be derived from full length antibodies or antibody fragments (*e.g.* $F(ab')_2$ bispecific antibodies). In accordance with the present invention, the BsAb may possess one arm which binds the WSX receptor and another arm which binds to a cytokine or another cytokine receptor (or a subunit thereof) such as the receptors for TPO, EPO, G-CSF, IL-4, IL-7, GH, PRL; the α or β subunits of the IL-3, GM-CSF, IL-5, IL-6, LIF, OSM and CNTF receptors; or the α, β or γ subunits of the IL-2 receptor complex. For example, the BsAb may bind both WSX receptor and gp130.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al., Nature* 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology* 121:210 (1986).

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. The following techniques can also be used for the production of bivalent antibody fragments which are not necessarily bispecific. According to these techniques, Fab'-SH fragments can be recovered from *E. coli,* which can be chemically coupled to form bivalent antibodies. Shalaby *et al., J. Exp. Med.* 175:217-225 (1992) describe the production of a fully

humanized BsAb F(ab')$_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the BsAb. The BsAb thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. See also Rodrigues *et al., Int. J. Cancers* (Suppl.) 7:45-50 (1992).

Various techniques for making and isolating bivalent antibody fragments directly from recombinant cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger *et al., Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making BsAb fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making BsAb fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.* 152:5368 (1994).

5. Antibody Screening

It may be desirable to select antibodies with a strong binding affinity for the WSX receptor. Antibody affinities may be determined by saturation binding; enzyme-linked immunoabsorbent assay (ELISA); and competition assays (*e.g.* RIA's), for example. The antibody with a strong binding affinity may bind the WSX receptor with a binding affinity (K_d) value of no more than about 1 x 10^{-7} M, preferably no more than about 1 x 10^{-8} M and most preferably no more than about 1 x 10^{-9} M (*e.g.* to about 1 x 10^{-12}M).

In another embodiment, one may screen for an antibody which binds a WSX receptor epitope of interest. For example, an antibody which binds to the epitope bound by antibody 2D7, 1G4, 1E11 or 1C11 (see Example 13) or antibody clone #3, #4 or #17 (see Example 14) can be identified. To screen for antibodies which bind to the epitope on WSX receptor bound by an antibody of interest (*e.g.,* those which block binding of any one of the above antibodies to WSX receptor), a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping,

e.g. as described in Champe *et al., J. Biol. Chem.* 270:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

In one particularly preferred embodiment of the invention, agonist antibodies are selected. Various methods for selecting agonist antibodies are available. In one embodiment, one evaluates the agonistic properties of the antibody upon binding to a chimeric receptor comprising the WSX receptor extracellular domain in an assay called the kinase receptor activation enzyme linked immunoadsorbent assay (KIRA ELISA) described in WO95/14930 (expressly incorporated herein by reference).

To perform the KIRA ELISA, a chimeric receptor comprising the extracellular domain of the WSX receptor and the transmembrane and intracellular domain of Rse receptor (Mark *et al.*, *Journal of Biological Chemistry* 269(14):10720-10728 (1994)) with a carboxyl-terminal herpes simplex virus glycoprotein D (gD) tag is produced and dp12.CHO cells are transformed therewith as described in Example 4 of WO95/14930.

The WSX/Rse.gD transformed dp12.CHO cells are seeded ($3x10^4$ per well) in the wells of a flat-bottom-96 well culture plate in 100μl media and cultured overnight at 37° C in 5% CO_2. The following morning the well supernatants are removed and various concentrations of the antibody are added to separate wells. The cells are stimulated at 37° C for 30 min., the well supernatants are decanted. To lyse the cells and solubilize the chimeric receptors, 100 μl of lysis buffer is added to each well. The plate is then agitated gently on a plate shaker (Bellco Instruments, Vineland, NJ) for 60 min. at room temperature.

While the cells are being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4° C with the 5B6 monoclonal anti-gD antibody (5.0 μg/ml in 50 mM carbonate buffer, pH 9.6, 100 μl/well) is decanted and blocked with 150 μl/well of Block Buffer for 60 min. at room temperature. After 60 minutes, the anti-gD 5B6 coated plate is washed 6 times with wash buffer (PBS containing 0.05 % TWEEN 20™ and 0.01 % thimerosal).

The lysate containing solubilized WSX/Rse.gD from the cell-culture microtiter well is transferred (85μl/well) to anti-gD 5B6 coated and blocked ELISA well and is incubated for 2 h at room temperature. The unbound WSX/Rse.gD is removed by washing with wash buffer and 100 μl of biotinylated 4G10 (anti-phosphotyrosine) diluted 1:18000 in dilution buffer (PBS containing 0.5 % BSA, 0.05 % Tween-20, 5 mM EDTA, and 0.01 % thimerosal), *i.e.* 56 ng/ml is added to each well. After incubation for 2 h at room temperature the plate is washed and HRPO-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) is added to each well. The plate is incubated for 30 minutes at room temperature with gentle agitation. The free avidin-conjugate is washed away and 100 μl freshly prepared substrate solution (tetramethyl benzidine (TMB); 2-component substrate kit;

Kirkegaard and Perry, Gaithersburg, MD) is added to each well. The reaction is allowed to proceed for 10 minutes, after which the color development is stopped by the addition of 100μl/well 1.0 M H_3PO_4. The absorbance at 450 nm is read with a reference wavelength of 650 nm ($ABS_{450/650}$), using a *vmax* plate reader (Molecular Devices, Palo Alto, CA) controlled with a Macintosh Centris 650 (Apple Computers, Cupertino, CA) and DeltaSoft software (BioMetallics, Inc, Princeton, NJ).

Those antibodies which have an IC50 in the KIRA ELISA of about 0.5μg/ml or less (*e.g.* from about 0.5μg/ml to about 0.001 μg/ml), preferably about 0.2μg/ml or less and most preferably about 0.1μg/ml or less are preferred agonists.

In another embodiment, one screens for antibodies which activate downstream signaling molecules for OB protein. For example, the ability of the antibody to activate Signal Transducers and Activators of Transcription (STATs) can be assessed. The agonist antibody of interest may stimulate formation of STAT-1 and STAT-3 complexes, for example. To screen for such antibodies, the assay described in Rosenblum *et al. Endocrinology* 137(11):5178-5181 (1996) may be performed.

Alternatively, an antibody which stimulates proliferation and/or differentiation of hematopoietic cells can be selected. For example, the hematopoiesis assays of Example 10 below can be performed. For example, murine fetal liver flASK stem cells may be isolated from the midgestational fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994) and studied in stem cell suspension culture or methylcellulose assays. For the stem cell suspension cultures, twenty thousand of the fLASK cells are seeded in individual wells in a 12 well format in DMEM 4.5/F12 media supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT) and L-glutamine. Growth factors are added at the following concentrations: kit ligand (KL) at 25 ng/mL, interleukin-3 (IL-3) at 25 ng/mL, interleukin-6 (IL-6) at 50 ng/mL, G-CSF at 100 ng/mL, GM-CSF at 100 ng/mL, EPO at 2U/mL, interleukin-7 (IL-7) at 100 ng/mL (all growth factors from R and D Systems, Minneapolis, MN). The agonist antibody is then added and the ability of the antibody to expand the flASK cells grown in suspension culture is assessed. Methylcellulose assays are performed as previously described (Zeiger *et al.*, *supra*). Briefly, methylcellulose colony assays are performed using "complete" methylcellulose or pre-B methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) with the addition of 25 ng/mL KL (R and D Systems, Minneapolis, MN). Cytospin analyses of the resultant colonies are performed as previously described in Zeigler *et al.* The ability of the agonist antibody to augment myeloid, lymphoid and erythroid colony formation is assessed. Also, the effect of the agonist antibody on the murine bone marrow stem cell population; $Lin^{lo}Sca^{+}$ may be evaluated.

One may select an agonist antibody which induces a statistically significant decrease in body weight and/or fat-depot weight and/or food intake in an obese mammal (*e.g.* in an *ob/ob* mouse). Methods for screening for such molecules are described in Levin *et al. Proc. Natl. Acad. Sci. USA* 93:1726-1730 (1996), for example. Preferred agonist antibodies are those which exert adipose-reducing effects in an obese mammal, such as the *ob/ob* mouse, which are in excess of those induced by reductions in food intake.

The antibody of interest herein may have the hypervariable region residues of one of the antibodies in Examples 13 and 14. Also, the invention encompasses "affinity matured" forms of these antibodies in which hypervariable region residues of these antibodies have been modified. Such affinity matured antibodies will preferably have a biological activity which is the same as or better than that of the original antibody. The affinity matured antibody may have from about 1-10, *e.g.* 5-10 deletions, insertions or substitutions (but preferably substitutions) in the hypervariable regions thereof. One useful procedure for generating affinity matured antibodies is called "alanine scanning mutagenesis" (Cunningham and Wells *Science* 244:1081-1085 (1989)). Here, one or more of the hypervariable region residue(s) are replaced by alanine or polyalanine residue(s) to affect the interaction of the amino acids with the WSX receptor. Those hypervariable region residue(s) demonstrating functional sensitivity to substitution are then refined by introducing further or other mutations at or for the sites of substitution. The ala-mutants produced this way are screened for their biological activity as described herein. Another procedure is affinity maturation using phage display (Hawkins *et al. J. Mol. Biol.* 254:889-896 (1992) and Lowman *et al. Biochemistry* 30(45):10832-10837 (1991)). Briefly, several hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody mutants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed mutants are then screened for their biological activity (*e.g.* binding affinity).

6. Antibody Modifications

It may be desirable to tailor the antibody for various applications. Exemplary antibody modifications are described here.

In certain embodiments of the invention, it may be desirable to use an antibody fragment, rather than an intact antibody. In this case, it may be desirable to modify the antibody fragment in order to increase its serum half-life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the antibody fragment. See WO96/32478 published October

17, 1996. Alternatively, the antibody may be conjugated to a nonproteinaceous polymer, such as those described above for the production of long half-life derivatives of OB protein.

Where the antibody is to be used to treat cancer for example, various modifications of the antibody (*e.g.* of a neutralizing antibody) which enhance the effectiveness of the antibody for treating cancer are contemplated herein. For example, it may be desirable to modify the antibody of the invention with respect to effector function. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al. Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al. Anti-Cancer Drug Design* 3:219-230 (1989).

The invention also pertains to immunoconjugates comprising the antibody described herein conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.,* a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugate antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be

prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.* avidin) which is conjugated to a cytotoxic agent (*e.g.* a radionucleotide).

The antibody may also be formulated as an immunoliposome. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl Acad. Sci. USA*, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.*81(19)1484 (1989).

The antibody of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (*e.g.* a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting

glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, *e.g.*, Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the antibody mutant by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, *e.g.*, Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

In other embodiments, the antibody can be covalently modified, with exemplary such modifications described above.

E. Therapeutic Uses for WSX Receptor Ligands and Antibodies

The WSX ligands (*e.g.* OB protein and anti-WSX receptor agonist antibodies) of the present invention are useful, in one embodiment, for weight reduction, and specifically, in the treatment of obesity, bulimia and other disorders associated with the abnormal expression or function of the OB and/or WSX receptor genes, other metabolic disorders such as diabetes, for reducing excessive levels of insulin in human patients (*e.g.* to restore or improve the insulin-sensitivity of such patients). Thus, these molecules can be used to treat a patient suffering from excessive food consumption and related pathological conditions such as type II adult onset diabetes, infertility (Chehab *et al. Nature Genentics* 12:318-320 (1996)), hypercholesterolemia, hyperlipidemia, cardiovascular diseases, arteriosclerosis, polycystic ovarian disease, osteoarthritis, dermatological disorders, insulin resistance, hypertriglyceridemia, cancer, cholelithiasis and hypertension.

In addition, the WSX ligands can be used for the treatment of kidney ailments, hypertension, and lung dysfunctions, such as emphysema.

In a further embodiment, the WSX ligands (such as agonist WSX receptor antibodies) of the present invention can be used to enhance repopulation of mature blood cell lineages in mammals having undergone chemo- or radiation therapy or bone marrow transplantation therapy. Generally, the ligands will act via an enhancement of the proliferation and/or differentiation (but especially proliferation) of primitive hematopoietic cells. The ligands may similarly be useful for treating

diseases characterized by a decrease in blood cells. Examples of these diseases include: anemia (including macrocytic and aplastic anemia); thrombocytopenia; hypoplasia; immune (autoimmune) thrombocytopenic purpura (ITP); and HIV induced ITP. Also, the ligands may be used to treat a patient having suffered a hemorrhage. WSX ligands may also be used to treat metabolic disorders such as obesity and diabetes mellitus, or to promote kidney, liver or lung growth and/or repair (*e.g.*, in renal failure).

The WSX receptor ligands and antibodies may be administered alone or in concert with one or more cytokines. Furthermore, as an alternative to adminstration of the WSX ligand protein, gene therapy techniques (discussed in the section above entitled "Therapeutic Uses for the WSX Receptor") are also contemplated herein.

Potential therapeutic applications for WSX receptor neutralizing antibodies include the treatment of metabolic disorders (such as cachexia, anorexia and other wasting diseases characterized by loss of appetite, diminished food intake or body weight loss), stem cell tumors and other tumors at sites of WSX receptor expression, especially those tumors characterized by overexpression of WSX receptor.

For therapeutic applications, the WSX receptor ligands and antibodies of the invention are administered to a mammal, preferably a human, in a physiologically acceptable dosage form, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The WSX receptor ligands and antibodies also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes or to the lymph, to exert local as well as systemic therapeutic effects.

Such dosage forms encompass physiologically acceptable carriers that are inherently non-toxic and non-therapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and PEG. Carriers for topical or gel-based forms of WSX receptor antibodies include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, PEG, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations. The WSX

receptor ligand or antibody will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the WSX receptor ligand or antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *supra* and Langer, *supra*, or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate (Sidman *et al.*, *supra*), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated WSX receptor antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release WSX receptor ligand or antibody compositions also include liposomally entrapped antibodies. Liposomes containing the WSX receptor ligand or antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Ordinarily, the liposomes are the small (about 200-800 Angstroms) unilamelar type in which the lipid content is greater than about 30 mol.% cholesterol, the selected proportion being adjusted for the optimal WSX receptor ligand or antibody therapy. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

For the prevention or treatment of disease, the appropriate dosage of WSX receptor ligand or antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibodies are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the WSX receptor ligand or antibody, and the discretion of the attending physician. The WSX receptor ligand or antibody is suitably administered to the patient at one time or over a series of treatments.

Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg of WSX receptor ligand or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 μg/kg (*e.g.* 1-50 μg/kg) or more, depending on the factors mentioned above. For example, the dose may be the same as that for other cytokines such as G-CSF, GM-CSF and EPO. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

When one or more cytokines are co-administered with the WSX receptor ligand, lesser doses of the WSX ligand may be employed. Suitable doses of a cytokine are from about 1μg/kg to about 15mg/kg of cytokine. A typical daily dosage of the cytokine might range from about 1 μg/kg to 100 μg/kg (*e.g.* 1-50 μg/kg) or more. For example, the dose may be the same as that for other cytokines such as G-CSF, GM-CSF and EPO. The cytokine(s) may be administered prior to, simultaneously with, or following administration of the WSX ligand. The cytokine(s) and WSX ligand may be combined to form a pharmaceutically composition for simultaneous administration to the mammal. In certain embodiments, the amounts of WSX ligand and cytokine are such that a synergistic repopulation of blood cells (or synergistic increase in proliferation and/or differentiation of hematopoietic cells) occurs in the mammal upon administration of the WSX ligand and cytokine thereto. In other words, the coordinated action of the two or more agents (*i.e.* the WSX ligand and cytokine(s)) with respect to repopulation of blood cells (or proliferation/differentiation of hematopoietic cells) is greater than the sum of the individual effects of these molecules.

For treating obesity and associated pathological conditions, the WSX ligand may be administered in combination with other compound(s) for combatting or preventing obesity. Substances useful for this purpose include, *e.g.*, hormones (catecholamines, glucagon, ACTH); clofibrate; halogenate; cinchocaine; chlorpromazine; appetite-suppressing drugs acting on noradrenergic neurotransmitters such as mazindol and derivatives of phenethylamine, *e.g.*, phenylpropanolamine, diethylpropion, phentermine, phendimetrazine, benzphetamine, amphetamine, methamphetamine, and phenmetrazine; drugs acting on serotonin neurotransmitters such as fenfluramine, tryptophan, 5-hydroxytryptophan, fluoxetine, and sertraline; centrally active drugs such as naloxone, neuropeptide-Y, galanin, corticotropin-releasing hormone, and cholecystokinin; a cholinergic agonist such as pyridostigmine; a sphingolipid such as a lysosphingolipid or derivative thereof (EP 321,287 published June 21, 1989); thermogenic drugs such as thyroid hormone, ephedrine, beta-adrenergic agonists; drugs affecting the gastrointestinal tract such as enzyme

inhibitors, *e.g.*, tetrahydrolipostatin, indigestible food such as sucrose polyester, and inhibitors of gastric emptying such as threo-chlorocitric acid or its derivatives; β-adrenergic agonist such as isoproterenol and yohimbine; aminophylline to increase the β-adrenergic-like effects of yohimbine, an α_2-adrenergic blocking drug such as clonidine alone or in combination with a growth hormone releasing peptide (U.S. Pat. No. 5,120,713 issued June 9, 1992); drugs that interfere with intestinal absorption such as biguanides such as metformin and phenformin; bulk fillers such as methylcellulose; metabolic blocking drugs such as hydroxycitrate; progesterone; cholecystokinin agonists; small molecules that mimic ketoacids; agonists to corticotropin-releasing hormone; an ergot-related prolactin-inhibiting compound for reducing body fat stores (U.S. Pat. No. 4,783,469 issued November 8, 1988); beta-3-agonists; bromocriptine; antagonists to opioid peptides; antagonists to neuropeptide Y; glucocorticoid receptor antagonists; growth hormone agonists; combinations thereof; *etc.* This includes all drugs described by Bray and Greenway, *Clinics in Endocrinol. and Metabol.*, 5:455 (1976).

These adjunctive agents may be administered at the same time as, before, or after the administration of WSX ligand and can be administered by the same or a different administration route than the WSX ligand.

The WSX ligand treatment may occur without, or may be imposed with, a dietary restriction such as a limit in daily food or calorie intake, as is desired for the individual patient.

F. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the conditions described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the WSX ligand. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container holding a cytokine for co-administration with the WSX ligand. Further container(s) may be provided with the article of manufacture which may hold, for example, a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution or dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

G. Non-Therapeutic Uses for WSX Receptor Ligands and Antibodies

WSX receptor ligands and antibodies may be used for detection of and/or enrichment of hematopoietic stem cell/progenitor cell populations in a similar manner to that in which CD34 antibodies are presently used. For stem cell enrichment, the WSX receptor antibodies may be utilized in the techniques known in the art such as immune panning, flow cytometry or immunomagnetic beads.

In accordance with one *in vitro* application of the WSX ligands, cells comprising the WSX receptor are provided and placed in a cell culture medium. Examples of such WSX-receptor-containing cells include hematopoietic progenitor cells, such as CD34+ cells.

Suitable tissue culture media are well known to persons skilled in the art and include, but are not limited to, Minimal Essential Medium (MEM), RPMI-1640, and Dulbecco's Modified Eagle's Medium (DMEM). These tissue culture medias are commercially available from Sigma Chemical Company (St. Louis, MO) and GIBCO (Grand Island, NY). The cells are then cultured in the cell culture medium under conditions sufficient for the cells to remain viable and grow in the presence of an effective amount of WSX ligand and, optionally, further cytokines and growth factors. The cells can be cultured in a variety of ways, including culturing in a clot, agar, or liquid culture.

The cells are cultured at a physiologically acceptable temperature such as 37° C, for example, in the presence of an effective amount of WSX ligand. The amount of WSX ligand may vary, but preferably is in the range of about 10 ng/ml to about 1mg/ml. The WSX ligand can of course be added to the culture at a dose determined empirically by those in the art without undue experimentation. The concentration of WSX ligand in the culture will depend on various factors, such as the conditions under which the cells and WSX ligand are cultured. The specific temperature and duration of incubation, as well as other culture conditions, can be varied depending on such factors as, *e.g.*, the concentration of the WSX ligand, and the type of cells and medium.

It is contemplated that using WSX ligand to enhance cell proliferation and/or differentiation *in vitro* will be useful in a variety of ways. For instance, hematopoietic cells cultured *in vitro* in the presence of WSX ligand can be infused into a mammal suffering from reduced levels of the cells. Also, the cultured hematopoietic cells may be used for gene transfer for gene therapy applications. Stable *in vitro* cultures can be also used for isolating cell-specific factors and for expression of endogenous or recombinantly introduced proteins in the cell. WSX ligand may also be used to enhance cell survival, proliferation and/or differentiation of cells which support the growth and/or differentiation of other cells in cell culture.

The WSX receptor antibodies of the invention are also useful as affinity purification agents. In this process, the antibodies against WSX receptor are immobilized on a suitable support, such

a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the WSX receptor to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the WSX receptor, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the WSX receptor from the antibody.

WSX receptor antibodies may also be useful in diagnostic assays for WSX receptor, *e.g.*, detecting its expression in specific cells, tissues, or serum. For diagnostic applications, antibodies typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, *e.g.*, ^{125}I, ^{32}P, ^{14}C, or ^{3}H; or an enzyme, such as alkaline phosphatase, beta-galactosidase, or horseradish peroxidase.

Any method known in the art for separately conjugating the polypeptide variant to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature* 144:945 (1962); David *et al.*, *Biochemistry* 13:1014 (1974); Pain *et al.*, *J. Immunol. Meth* 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.* 30:407 (1982).

The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of WSX receptor in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, *e.g.*, US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable

moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

H. Deposit of Materials

The following biological materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

Deposit Designation	ATCC No.	Deposit Date
Baf3/WSX E63x7 sort (Baf3 cells expressing human WSX receptor variant 13.2)	ATCC CRL 12015	Jan 10, 1996
2D7 hybridoma cell line		
1G4 hybridoma cell line	HB-12243	Dec 11, 1996
1E11 hybridoma cel l line		
1C11 hybridoma cell line		

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture for 30 years from the date of deposit. Each of the deposited cultures will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures (a) that access to the culture will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR §1.14 and 35 USC §122, and (b) that all restrictions on the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent.

The assignee of the present application has agreed that if any of the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, it will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited cell lines is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by any culture deposited, since the deposited embodiment is intended as an illustration of one aspect of the invention and any culture that is functionally equivalent is within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustration that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

III. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

The disclosures of all publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

EXAMPLE 1

Cloning of Human WSX Receptor

An oligonucleotide probe designated WSX.6 #1 was synthesized based upon the T73849 EST sequence. The WSX.6 #1 probe was a 51mer having the following sequence:
5' GTCAGTCTCCCAGTTCCAGACTTGTGTGCAGTCTATGCTGTTCAGGTGCGC - 3' (SEQ ID NO:45).

The radiolabeled WSX.6 #1 probe was used to probe 1.2 x 10^6 clones from a random and oligo dT primed λgt10 fetal liver library (Clontech, Palo Alto, CA). Following hybridization at 42° C overnight, the filters were washed at 50° C in 0.5 x SSC and 0.1% $NaDodSO_4$ (SDS). From the initial screen, 10 clones were selected and upon subsequent screening 5 individual plaque pure clones were isolated. Of these 5 individual clones, four clones designated 1, 5, 6 and 9 were subcloned into pBSSK$^-$ (Stratagene) following EcoRI digestion. Sequence analysis revealed clone 5 and clone 9 contained the putative initiation methionine and signal peptide. Clone 6 (designated 6.4) contained the most 3' end sequence and subsequently was used for further screening.

To obtain the full length gene, clone 6.4 (fragment Nsi-Hind III) was radiolabeled and used to screen 1.2 x 10^6 clones from a λgt 10 library constructed from a hepatoma Hep3B cell line. This

screen resulted in 24 positive clones. Following PCR analysis of the clones using λgt10 primers (F and R), the four longest clones 12.1, 13.2, 22.3, and 24.3 were isolated. These clones were subcloned into pBSSK$^-$ using the EcoRI site, and following examination by restriction enzyme digest, clones 12.1 and 13.2 were submitted for sequencing. DNA sequencing was performed with the Taq dye deoxynucleotide terminator cycle sequencing kit on an automated Applied Biosystems DNA sequencer.

The assembled contiguous sequence from all the isolated clones encoded a consensus amino terminus for the newly identified polypeptide designated the WSX receptor. However, sequence analysis revealed that at least three naturally occurring variants of the WSX receptor exist which have different cytoplasmic regions. These variants appear to be differentially spliced at the lysine residue at position 891. Clone 6.4 stops 5 amino acids after Lys 891. Clone 12.1 is different from 13.2 and 6.4 following Lys 891 and encodes a putative box 2 region which is distinct from that encoded by clone 13.2. Clone 13.2 contains a potential box 1 region and following Lys 891 encodes putative box 2 and box 3 motifs. See, Baumann *et al., Mol. Cell. Biol.* 14(1):138-146 (1994).

The full length WSX gene based on the clone 13.2 cytoplasmic region putatively encodes an 1165 amino acid transmembrane protein. The 841 amino acid extracellular domain (ECD) contains two WSXWS domains. The ECD is followed by a 24 amino acid transmembrane domain and a 300 amino acid cytoplasmic region.

EXAMPLE 2

WSX Receptor Immunoadhesin

Using polymerase chain amplification, a WSX receptor immunoadhesin was created by engineering an in-frame fusion of the WSX receptor gene extracellular domain (WSX.ECD) with human CH2CH3(Fc)IgG (Bennett *et al., J.Biol. Chem.* 266(34):23060-23067 (1991)) at the C terminus of the ECD and cloned into pBSSK$^-$(Stratagene). For expression, the WSX-Fc was excised with ClaI and BstEII and ligated into the pRK5.HuIF.grbhIgG Genenase I vector (Beck *et al., Molecular Immunology* 31(17):1335-1344 (1994)), to create the plasmid pRK5.WSX-IgG Genenase I. This plasmid was transiently transfected into 293 cells using standard calcium phosphate transfection techniques. The transfected cells were cultured at 37°C in 5% CO_2 in DMEM F12 50:50 supplemented with 10% FBS, 100mM HEPES (pH 7.2) and 1mM glutamine. The WSX receptor immunoadhesin was purified using a ProSepA™ protein A column.

EXAMPLE 3

Antibody Production

In order to raise antibodies against the WSX receptor, the WSX receptor immunoadhesin of Example 2 was used to inoculate rabbits to raise polyclonal antibodies and mice to raise monoclonal antibodies using conventional technology.

EXAMPLE 4

Generation of a Cell Line Expressing WSX Receptor

The nucleic acid encoding full length WSX receptor variant 13.2 was inserted in the pRKtkNeo plasmid (Holmes *et al., Science* 253:1278-1280 (1991)). 100 μgs of the pRKtkNeo.WSX plasmid thus generated was linearized, ethanol precipitated and resuspended in 100 μL of RPMI 1640. 7×10^6 Baf3 cells (5×10^5/ml) were suspended in 900 μL of RPMI and added to the linearized plasmid. Following electroporation at 325V, 1180 μF using a BRL electroporation apparatus, the cells were plated into 15 mls of RPMI 1640 containing 5% WEHI3B conditioned media and 15% serum. 48 hours later cells were selected in 2mg/ml G418.

To obtain the Baf3/WSX cell line expressing WSX receptor variant 13.2, the G418 selected clones were analyzed by FACS using the rabbit polyclonal antisera raised against the WSX-Fc chimeric protein as described above. The highest expressing clone (designated E6) was sorted by FACS to maintain a population with a high level of WSX receptor expression.

EXAMPLE 5

Role of WSX Receptor in Cellular Proliferation

The proliferative potentials of WSX receptor variants 13.2 and 12.1 were tested by constructing human growth hormone receptor-WSX receptor (GH-WSX) fusions encoding chimeric proteins consisting of the GH receptor extracellular and transmembrane domains and the WSX receptor variant 13.2 or 12.1 intracellular domains. These chimeric gene fusions were transfected into the IL-3 dependent cell line Baf3. The ability of the GH-WSX transfected Baf3 cells to respond to exogenous growth hormone (GH) was tested in a thymidine incorporation assay. As can be seen in Figs. 6 and 8, the GH-WSX receptor variant 13.2 chimera was capable of increasing thymidine uptake in the transfected Baf3 cells, thus indicating the proliferative potential of the WSX receptor variant 13.2. However, WSX receptor variant 12.1 was unable to transmit a proliferative signal in this experiment (Fig. 8).

Materials and Methods

Recombinant PCR was used to generate the chimeric receptors containing the extracellular and transmembrane domains of the hGH receptor and the cytoplasmic domain of either WSX receptor variant 12.1 or variant 13.2. In short, the cytoplasmic domain of either variant 12.1 or 13.2 beginning with Arg at amino acid 866 and extending down to amino acid 958 or amino acid 1165 respectively, was fused in frame, by sequential PCR, to the hGH receptor extracellular and transmembrane domain beginning with Met at amino acid 18 and extending down to Arg at amino acid 274. The GH-WSX chimera was constructed by first using PCR to generate the extracellular and transmembrane domain of the human GH receptor. The 3' end primer used for this PCR contained 20 nucleotides at the 5' end of the primer corresponding to the first 20 nucleotides of the WSX cytoplasmic domain. The 3' end of the chimera was generated using PCR where the 5' end primer contained the last 19 nucleotides of the human GH receptor transmembrane domain. To generate the full length chimera, the 5' end of the human GH receptor product was combined with the 3' end WSX receptor cytoplasmic PCR product and subsequently amplified to create a fusion of the two products.

This chimeric fusion was digested with ClaI and XbaI and ligated to pRKtkNeo (Holmes *et al., Science* 253:1278-1280 (1991)) to create the chimeric expression vector. The IL-3 dependent cell line Baf3 was then electroporated with this hGH/WSX chimeric expression vector.

Briefly, 100µg of the pRKtkNeo/GH.WSX plasmid was linearized, ethanol precipitated and resuspended in 100 µL of RPMI 1640. 7×10^6 Baf3 cells (5×10^5/ml) were suspended in 900 µL of RPMI and added to the linearized plasmid. Following electroporation at 325V, 1180 µF using a BRL electroporation apparatus, the cells were plated into 15 mls of RPMI 1640 containing 5% wehi conditioned media and 15% serum. 48 hours later, cells were selected in 2mg/ml G418.

To obtain the Baf3/GH.WSX cell lines, the G418 selected cells were FACS sorted using an anti-human GH mAb (3B7) at 1µg/ml. The top 10% expressing cells were selected and expanded.

EXAMPLE 6

Expression Analysis of the WSX Receptor

The expression profile of the WSX receptor was initially examined by Northern analysis. Northern blots of human fetal or adult tissue mRNA were obtained from Clontech (Palo Alto, California). A transcript of approximately 6 kb was detected in human fetal lung, liver and kidney. In the adult, low level expression was detected in a variety of tissues including liver, placenta, lung skeletal muscle, kidney, ovary, prostate and small intestine.

PCR analysis of human cord blood identified transcripts in CD34$^+$ subfraction. By PCR analysis, all three variants of the WSX receptor were present in CD34$^+$ cells. The CD34$^-$ subfraction appeared negative by this same PCR analysis.

By PCR analysis, both the 6.4 variant and 13.2 variant were evident in the AA4$^+$Sca$^+$Kit$^+$ (flASK) cell population isolated from the mid-gestation fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994). No clones containing the 12.1 variant cytoplasmic tail have been isolated from murine tissues.

Human B cells isolated from peripheral blood using anti-CD19/20 antibodies were also positive for short form (6.4 variant) and long from (13.2 variant) receptor mRNA expression.

The WSX receptor appears to be expressed on both progenitor and more mature hematopoietic cells.

EXAMPLE 7

Cloning of Murine WSX Receptor

The human WSX receptor was used as a probe to isolate murine WSX receptor. The pRKtkNeo.WSX plasmid of Example 4 was digested using Ssp1. This Ssp1 fragment (1624 bps) was isolated, and radiolabelled, and used to screen a murine liver λgt10 library (Clontech). This resulted in 4 positive clones which were isolated and sequenced after sub-cloning into pBSSK$^-$ via EcoRI digestion. The resultant clones, designated 1, 2, 3, 4 showed homology to the extracellular domain of the human WSX receptor; the contiguous sequences resulting from these clones extended from the initiation methionine to tryptophan at position 783. The overall similarity of human WSX receptor and murine WSX receptor is 73 % over this region of the respective extracellular domains (see Figs. 4A-B).

EXAMPLE 8

The Role of WSX Receptor in Hematopoietic Cell Proliferation

The presence of the WSX receptor in the enriched human stem cell population CD34$^+$ from cord blood is indicative of a potential role for this receptor in stem cell/progenitor cell proliferation.

The proliferation of CD34$^+$ human blood cells in methylcellulose media (Stem Cell Technologies) was determined in the presence or absence of WSX receptor antisense oligonucleotides. These experiments were also repeated in the murine hematopoietic system using AA4$^+$ Sca$^+$ Kit$^+$ stem cells from the murine fetal liver. In both instances, the antisense oligonucleotides statistically significantly inhibited colony formation from the hematopoietic progenitor

cells. See Table 1 below. The anti-proliferative effects were most pronounced using the -20 antisense and the +85 antisense oligonucleotide constructs. This inhibition was not lineage specific to any particular myeloid lineage that resulted from the progenitor expansion. The principal effect of the antisense oligonucleotides was a reduction of overall colony numbers. The size of the individual colonies was also reduced.

Antisense oligonucleotide experiments using both human and murine stem cells demonstrated an inhibition of myeloid colony formation. Although, the reduction in myelopoiesis observed in these assays could be prevented by the additional inclusion of G-CSF and GM-CSF in the culture medium. These data serve to illustrate the redundancy of cytokine action in the myelopoietic compartment.

TABLE 1

EXPERIMENT	OLIGO	AVG. COLONY #	% INHIBITION
Human Cord Blood (KL)	(-20)AS	32	
	(-20)S	100	70
	(-20)SCR	114	
	(+85)AS	80	
	(+85)S	123	38
	(+85)SCR	138	
	Control	158	
Human Cord Blood	(-20)AS	78	
(IL-3, IL-6, KL)	(-20)S	188	54
	(-20)SCR	151	
	(+85)AS	167	
	(+85)S	195	18
	(+85)SCR	213	
	Control	266	
Human Cord Blood (KL)	(-20)AS	42	
	(-20)S	146	69
	(-20)SCR	121	
	(+85)AS	123	
	(+85)S	162	23
	(+85)SCR	156	
	Control	145	
Murine Fetal Liver (KL)	(+84)AS	33	
	(+84)S	86	54
	(+84)SCR	57	
	(-20)AS	27	
	(-20)S	126	71
	(-20)SCR	60	
	(-99)AS	109	

EXPERIMENT	OLIGO	AVG. COLONY #	% INHIBITION
	(-99)S	93	0
	(-99)SCR	109	
	Control	121	
Murine Fetal Liver (KL)	(-213)AS	51	
	(-213)S	60	10
	(-213)SCR	53	
	(+211)AS	58	
	(+211)S	54	3
	(+211)SCR	66	
	Control	59	

Materials and Methods

Human stem cells: Human umbilical cord blood was collected in PBS/Heparin (1000μ/ml). The mononuclear fraction was separated using a dextran gradient and any remaining red blood cells lysed in 20 mM NH_4 Cl. $CD34^+$ cells were isolated using $CD34^+$ immunomagnetic beads (Miltenyi, CA). These isolated $CD34^+$ cells were found to be 90-97% $CD34^+$ by FACS analysis.

Murine stem cells: Midgestation fetal liver were harvested and positively selected for the $AA4^-$ antigen by immune panning. The $AA4^-$ positive fraction was then further enriched for stem cell content by FACS isolation of the $AA4^+$ Sca^+ Kit^+ fraction.

Antisense experiments: Oligodeoxynucleotides were synthesized against regions of the human or murine WSX receptors. For each oligonucleotide chosen, antisense (AS), sense (S) and scrambled (SCR) versions were synthesized (see Fig. 7). + or - indicates position relative the initiation methionine of the WSX receptor. $CD34^+$ or $AA4^+$ Sca^+ Kit^+ cells were incubated at a concentration of 10^3/ml in 50:50 DMEM/F12 media supplemented with 10% FBS, L-glutamine, and GIBCO™ lipid concentrate containing either sense, antisense or scrambled oligonucleotides at a concentration of 70 μg/ml. After 16 hours, a second aliquot of the respective oligonucleotide was added (35 μg/ml) and the cells incubated for a further 6 hours.

Colony assays: 5000 cells from each of the above conditions were aliquoted into 5 ml of methylcellulose (Stem Cell Technologies) containing kit ligand (KL) (25 ng/ml), interleukin-3 (IL-3) (25 ng/ml) and interleukin-6 (IL-6) (50 ng/ml). The methylcellulose cultures were then incubated at 37°C for 14 days and the resultant colonies counted and phenotyped. All assays were performed in triplicate.

EXAMPLE 9

WSX Receptor Variant 13.2 is a Receptor for OB Protein

The WSX receptor variant 13.2 has essentially the same amino acid sequence as the recently cloned leptin (OB) receptor. See Tartaglia *et al.*, *Cell* 83:1263-1271 (1995). OB protein was able to stimulate thymidine incorporation in Baf3 cells transfected with WSX receptor variant 13.2 as described in Example 4 (See Fig. 9).

OB protein expression in hematopoietic cells was studied. Oligonucleotide primers designed specifically against the OB protein illustrated the presence of this ligand in fetal liver and fetal brain as well as in two fetal liver stromal cell lines, designated 10-6 and 7-4. Both of these immortalized stromal cell lines have been demonstrated to support both myeloid and lymphoid proliferation of stem cell populations (Zeigler *et al.*, *Blood* 84:2422-2430 (1994)).

EXAMPLE 10

Role of OB Protein in Hematopoiesis

To examine the hematopoietic activity of OB protein, a variety of *in vitro* assays were performed.

Murine fetal liver flASK stem cells were isolated from the midgestational fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994) and studied in stem cell suspension culture or methylcellulose assays.

For the stem cell suspension cultures, twenty thousand of the fLASK cells were seeded in individual wells in a 12 well format in DMEM 4.5/F12 media supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT) and L-glutamine. Growth factors were added at the following concentrations: kit ligand (KL) at 25 ng/mL, interleukin-3 (IL-3) at 25 ng/mL, interleukin-6 (IL-6) at 50 ng/mL, G-CSF at 100 ng/mL, GM-CSF at 100 ng/mL, EPO at 2U/mL, interleukin-7 (IL-7) at 100 ng/mL (all growth factors from R and D Systems, Minneapolis, MN). OB protein was added at 100 ng/mL unless indicated otherwise. Recombinant OB protein was produced as described in Levin *et al.*, *Proc. Natl. Acad. Sci. (USA)* 93:1726-1730 (1996).

In keeping with its ability to transduce a proliferative signal in Baf3 cells (see previous Example), OB protein dramatically stimulated the expansion of flASK cells grown in suspension culture in the presence of kit ligand (Fig. 10A). The addition of OB protein alone to these suspension cultures was unable to effect survival of the hematopoietic stem cells (HSCs). When a variety of

hematopoietic growth factors in suspension culture assays were tested, the main synergy of OB protein appeared to be with KL, GM-CSF and IL-3 (Table 2). No preferential expansion of any particular lineage was observed from cytospin analysis of the resultant cultures.

TABLE 2

Factor	KL	KL+OB protein	OB protein
N/A	128+/-9	192+/-13	
G-CSF	131+/-3	177+/-8	30+/-5
GM-CSF	148+/-4	165+/-6	134+/-10
IL-3	189+/-7	187+/-4	144+/-
IL-6	112+/-4	198+/-5	32+/-3
EPO	121+/-3	177+/-8	30+/-6
IL-3 & IL-6	112+/-12	198+/-7	32+/-7

flASK stem cells were isolated. Twenty thousand cells were plated in suspension culture with the relevant growth factor combination. Cells were harvested and counted after 7 days. Cell numbers are presented x10^3. Assays were performed in triplicate and repeated in two independent experiments.

Methylcellulose assays were performed as previously described (Zeiger *et al.*, *supra*). Briefly, methylcellulose colony assays were performed using "complete" methylcellulose or pre-B methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) with the addition of 25 ng/mL KL (R and D Systems, Minneapolis, MN). Cytospin analyses of the resultant colonies were performed as previously described in Zeigler *et al.*

When these methylcellulose assays were employed, OB protein augmented myeloid colony formation and dramatically increased lymphoid and erythroid colony formation (Figs. 10B and 10C) which demonstrates that OB protein can act on very early cells of the hematopoietic lineage. Importantly, the hematopoietic activity of OB protein was not confined to fetal liver stem cells, the murine bone marrow stem cell population; $Lin^{lo}Sca^{+}$ also proliferated in response to OB protein (KL: 5 fold expansion, KL and OB protein: 10 fold expansion).

Further hematopoietic analysis of the role of the WSX receptor was carried out by examining hematopoietic defects in the *db/db* mouse.

These defects were assessed by measuring the proliferative potential of *db/db* homozygous mutant marrow. Under conditions favoring either myeloid (Humphries *et al., Proc. Natl. Acad. Sci. (USA)* 78:3629-3633 (1981)) or lymphoid (McNiece *et al., J. Immunol.* 146:3785-90 (1991)) expansion, the colony forming potential of the *db/db* marrow was significantly reduced when compared to the wild-type control marrow (Fig. 11). This was particularly evident when the comparison was made under pre-B methylcellulose conditions where KL and IL-7 are used to drive lymphopoiesis (McNiece *et al., supra*). Corresponding analysis of the complementary mouse mutation *ob/ob*, which is deficient in the production of OB protein (Zhang *et al., Nature* 372:425-431 (1994)), also indicated that the lymphoproliferative capacity is compromised in the absence of a functional OB protein signalling pathway (Fig. 11). However, this reduction was less than the reduction observed using *db/db* marrow.

Analysis of the cellular profile of the *db/db* and wild-type marrow revealed significant differences between the two. Overall cellularity of the *db/db* marrow was unchanged. However, when various B cell populations in the *db/db* marrow were examined, both decreased levels of $B220^+$ and $B220^+/CD43^+$ cells were found. $B220^+$ cells represent all B cell lineages while CD43 is considered to be expressed preferentially on the earliest cells of the B cell hierarchy (Hardy *et al., J. Exp. Med.* 173:1213-25 (1991)). No differences were observed between the CD4/CD8 staining profiles of the two groups. The TER119 (a red cell lineage marker) population was increased in the *db/db* marrow (Fig. 12A).

Comparison of the spleens from the two groups revealed a significant decrease in both tissue weight and cellularity of the *db/db* mice compared to the homozygote misty gray controls (0.063 ±0.009 g vs. 0.037±0.006 g and $1.10 \times 10^7 \pm 1 \times 10^4$ vs. $4.3 \times 10^6 \pm 10^3$ cells > p0.05). This decreased cellularity in the *db* spleen was reflected in a marked reduction in TER119 staining (Fig. 12B). This result appears to confirm the synergy demonstrated between OB protein and EPO and points to a role for OB protein in the regulation of erythropoiesis.

Examination of the hematopoietic compartment of the *db/db* mouse *in vivo* demonstrated a significant reduction in peripheral blood lymphocytes when compared to heterozygote or wild-type controls. *Db/db* mice fail to regulate blood glucose levels and become diabetic at approximately 6-8 weeks of age; therefore, peripheral blood counts as the animals matured were followed.

For procurement of blood samples, prior to the experiment and at time points throughout the study, 40 µL of blood was taken from the orbital sinus and immediately diluted into 10 mL of diluent

to prevent clotting. The complete blood count from each blood sample was measured on a Serrono Baker system 9018 blood analyzer within 60 min. of collection. Only half the animals in each dose group were bled on any given day, thus, each animal was bled on alternate time points. Blood glucose levels were measured in orbital sinus blood samples using One Touch glucose meters and test strips (Johnson and Johnson). The results of this experiment are shown in Figs. 13A-C.

This analysis demonstrated that peripheral blood lymphocytes are significantly reduced at all time points compared to control animals and that the peripheral lymphocyte population of the *db/db* mouse does not change significantly with age. FACS analysis revealed that the decreased lymphocyte population represented a decrease in both $B220^+$ cells and CD4/CD8 cells. Both erythrocyte and platelets are at wild-type levels throughout all time periods examined. The peripheral blood lymphocyte levels in *ob/ob* homozygous mutant mice were unchanged from wild-type controls.

Hematopoietic analysis of the *db/db* mouse can be complicated by the onset of diabetes. Therefore, the impact of high glucose levels on lymphopoiesis was examined by comparing the peripheral blood profiles and blood glucose levels in two other diabetic models, the glucokinase knockout heterozygote mouse (Grupe *et al.*, *Cell* 83:69-78 (1995)) and the IFN-α transgenic mouse (Stewart *et al.*, *Science* 260:1942-6 (1993)). Comparison of peripheral lymphocytes and blood glucose in *db/db* mice, their appropriate controls and the high glucose models illustrated no relationship between blood-glucose and lymphocyte counts (Fig. 14). These results suggest therefore that the lymphoid defects observed in the *db/db* mouse are directly attributed to the hematopoietic function of the OB protein signalling pathway.

To test the capacity of the *db/db* hematopoietic compartment to respond to challenge, the *db/db* mice and controls were subjected to sub-lethal irradiation C57BLKS/J *db/db*, C57BLKS/Jm^+/db, and C57BLKS/J $^+m/^+m$ mice were subjected to sub-lethal whole body irradiation (750 cGy, 190 cGy/min) as a single dose from a ^{137}Cs source. Ten animals were used per experimental group. The kinetics of hematopoietic recovery were then followed by monitoring the peripheral blood during the recovery phase. This experiment illustrated the inability of the *db/db* hematopoietic system to fully recover the lymphopoietic compartment of the peripheral blood 35 days post-irradiation. Platelet levels in these mice followed the same recovery kinetics as controls, however the reduction in erythrocytes lagged behind controls by 7-10 days. This finding may reflect the increased TER 119 population found in the marrow of the *db/db* mice (Fig. 12A).

Materials and Methods

Bone marrow, spleens and peripheral blood was harvested from the diabetic mouse strains: C57BLKS/J *db/db* (mutant), C57BLKS/J m+/db (lean heterozygote control littermate), C57BLKS/J+m/+m (lean homozygote misty gray coat control littermate) and the obese mouse strains: *C57BL*/6J-*ob/ob* (mutant) and the C57BL/6J-ob/+ (lean littermate control). All strains from the Jackson Laboratory, Bar Harbor, ME. A minimum of five animals were used per experimental group. Femurs were flushed with Hank's balanced salt solution (HBSS) plus 2% FCS and a single cell suspension was made of the bone marrow cells. Spleens were harvested and the splenic capsule was ruptured and filtered through a nylon mesh. Peripheral blood was collected through the retro-orbital sinus in phosphate buffered saline (PBS) with 10U/mL heparin and lmmol EDTA and processed as previously described. The bone marrow, splenocytes and peripheral blood were then stained with the monoclonal antibodies against the following antigens: B220/CD45R (Pan B cell) FITC antimouse, TER-119/erythroid cell R-PE antimouse, CD4 (L3T4), FITC antimouse, CD8 (Ly 3.2), FITC antimouse, and sIgM (Igh-6b), FITC antimouse (All monoclonals from Pharmigen, San Diego, CA). The appropriate isotype controls were included in each experiment. For methylcellulose assays, the bone marrow from five animals per group was pooled and 100,000 cell aliquots from each group used for each assay point.

EXAMPLE 11

Expression of OB-immunoadhesin

Using protein engineering techniques, the human OB protein was expressed as a fusion with the hinge, CH2 and CH3 domains of IgG1. DNA constructs encoding the chimera of the human OB protein and IgG1 Fc domains were made with the Fc region clones of human IgG1. Human OB cDNA was obtained by PCR from human fat cell dscDNA (Clontech Buick-Clone cDNA product). The source of the IgG1 cDNA was the plasmid pBSSK-CH2CH3. The chimera contained the coding sequence of the full length OB protein (amino acids 1-167 in Figure 16) and human IgG1 sequences beginning at aspartic acid 216 (taking amino acid 114 as the first residue of the heavy chain constant region (Kabat *et al.*, *Sequences of Proteins of Immunological Interest* 4th ed. (1987)), which is the first residue of the IgG1 hinge after the cysteine residue involved in heavy-light chain bonding, and ending with residues 441 to include the CH2 and CH3 Fc domains of IgG1. There was an insert of codons for three amino acids (GlyValThr) between the OB protein and IgG1 coding sequences. If

necessary, this short linker sequence can easily be deleted, for example by site directed deletion mutagenesis, to create an exact junction between the coding sequences of the OB protein and the IgG1 hinge region. The coding sequence of the OB-IgG1 immunoadhesin was subcloned into the pRK5-based vector pRK5tk-neo which contains a neomycine selectable marker, for transient expression in 293 cells using the calcium phosphate technique (Suva *et al.*, *Science* 237:893-896 (1987)). 293 cells were cultured in HAM's : Low Glucose DMEM medium (50:50), containing 10% FBS and 2 mM L-Gln. For purification of OB-IgG1 chimeras, cells were changed to serum free production medium PS24 the day after transfection and media collected after three days. The culture media was filtered.

The filtered 293 cell supernatant (400 ml) containing recombinant human OB-IgG1 was made 1 mM in phenylmethylsulfonyl fluoride and 2 µg/ml in aprotinin. This material was loaded at 4°C onto a 1 x 4.5 cm Protein A agarose column (Pierce catalog # 20365) equilibrated in 100 mM HEPES pH 8. The flow rate was 75 ml/h. Once the sample was loaded, the column was washed with equilibration buffer until the A_{280} reached baseline. The OB-IgG1 protein was eluted with 3.5 M $MgCl_2$ + 2% glycerol (unbuffered) at a flow rate of 15 ml/h. The eluate was collected with occasional mixing into 10 ml of 100 mM HEPES pH 8 to reduce the $MgCl_2$ concentration by approximately one-half and to raise the pH. The eluted protein was then dialyzed into phosphate buffered saline, concentrated, sterile filtered and stored either at 4°C or frozen at -70 °C. The OB-IgG1 immunoadhesin prepared by this method is estimated by SDS-PAGE to be greater than 90% pure.

EXAMPLE 12

Preparation of PEG-OB

The PEG derivatives of the human OB protein were prepared by reaction of hOB protein purified by reverse phase chromatography with a succinimidyl derivative of PEG propionic acid (SPA-PEG) having a nominal molecular weight of 10 kD, which had been obtained from Shearwater Polymers, Inc. (Huntsville, AL). After purification of the hOB protein by reverse phase chromatography, an approximately 1-2 mg/ml solution of the protein in 0.1% trifluoroacetic acid and approximately 40% acetonitrile, was diluted with 1/3 to 1/2 volume of 0.2 M borate buffer and the pH adjusted to 8.5 with NaOH. SPA-PEG was added to the reaction mixture to make 1:1 and 1:2 molar ratios of protein to SPA-PEG and the mixture was allowed to incubate at room temperature for one hour. After reaction and purification by gel electrophoresis or ion exchange chromatography, the

samples were extensively dialyzed against phosphate-buffered saline and sterilized by filtration through a 0.22 micron filter. Samples were stored at 4°C. Under these conditions, the PEG-hOB resulting from the 1:1 molar ratio protein to SPA-PEG reaction consisted primarily of molecules with one 10 kD PEG attached with minor amounts of the 2 PEG-containing species. The PEG-hOB from the 1:2 molar reaction consisted of approximately equal amounts of 2 and 3 PEGs attached to hOB, as determined by SDS gel electrophoresis. In both reactions, small amounts of unreacted protein were also detected. This unreacted protein can be efficiently removed by the gel filtration or ion exchange steps as needed. The PEG derivatives of the human OB protein can also be prepared essentially following the aldehyde chemistry described in EP 372,752 published June 13, 1990.

EXAMPLE 13

Murine Agonist Antibodies

Mice were immunized five times with 20μg of the WSX receptor immunoadhesin (see Example 2 above) resuspended in MPL-TDM (monophosphoryl lipid A/trehalose dicorynomycolate; Rabi, Immunochemical Research Inc.) into each foot pad. Three days after the last immunization, popliteal lymphoid cells were fused with mouse myeloma cells, X63-Ag8.8.653 cells, using 50% polyethylene glycol as described (Laskov *et al. Cell. Immunol.* 55:251 (1980)).

The initial screening of hybridoma culture supernatants was done using a capture ELISA. For the capture ELISA, microtiter plates (Maxisorb; Nunc, Kamstrup, Denmark) were coated with 50μl/well of 2μg/ml of goat antibodies specific to the Fc portion of human IgG (Goat anti-hIgG-Fc; Cappel), in PBS, overnight at 4°C and blocked with 2x BSA for 1 hr at room temperature. Then, 50μl/well of 2μg/ml of WSX receptor immunoadhesin was added to each well for 1 hr. The remaining anti-Fc binding sites were blocked with PBS containing 3% human serum and 10μg/ml of CD4-IgG for 1 hr. Plates were incubated with 50μl/well of 2μg/ml of anti-WSX receptor monoclonal antibody (or hybridoma culture supernatant) for 1 hr. Plates were then incubated with 50μl/well of HRP-goat anti-mouse IgG. The bound enzyme was detected by the addition of the substrate (OPD) and the plates were read at 490nM with an ELISA plate reader. Between each step, plates were washed in wash buffer (PBS containing 0.05% TWEEN 20™).

Agonist antibodies were screened for using the KIRA ELISA described in WO95/14930. A chimeric receptor comprising the extracellular domain of the WSX receptor and the transmembrane and intracellular domain of Rse receptor (Mark *et al., Journal of Biological Chemistry* 269(14):10720-

10728 (1994)) with a carboxyl-terminal herpes simplex virus glycoprotein D (gD) tag was produced and dp12.CHO cells were transformed therewith as described in Example 4 of WO95/14930.

The WSX/Rse.gD transformed dp12.CHO cells were seeded ($3x10^4$ per well) in the wells of a flat-bottom-96 well culture plate in 100µl media and cultured overnight at 37°C in 5% CO_2. The following morning the well supernatants were removed and various concentrations of purified mAb were then added to separate wells. The cells were stimulated at 37°C for 30 min. and the well supernatants were decanted. To lyse the cells and solubilize the chimeric receptors, 100 µl of lysis buffer was added to each well. The plate was then agitated gently on a plate shaker (Bellco Instruments, Vineland, NJ) for 60 min. at room temperature.

While the cells were being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4°C with the 5B6 monoclonal anti-gD antibody (5.0 µg/ml in 50 mM carbonate buffer, pH 9.6, 100 µl/well) was decanted and blocked with 150 µl/well of Block Buffer containing 2% BSA for 60 min. at room temperature. After 60 minutes, the anti-gD 5B6 coated plate was washed 6 times with wash buffer (PBS containing 0.05 % TWEEN 20™ and 0.01 % thimerosal).

The lysate containing solubilized WSX/Rse.gD from the cell-culture microtiter well was transferred (85µl/well) to anti-gD 5B6 coated and blocked ELISA well and was incubated for 2 h at room temperature. The unbound WSX/Rse.gD was removed by washing with wash buffer and 100 µl of biotinylated 4G10 (anti-phosphotyrosine) diluted 1:18000 in dilution buffer (PBS containing 0.5 % BSA, 0.05 % Tween-20, 5 mM EDTA, and 0.01 % thimerosal), *i.e.* 56 ng/ml was added to each well. After incubation for 2 h at room temperature the plate was washed and HRPO-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) was added to each well. The plate was incubated for 30 minutes at room temperature with gentle agitation. The free avidin-conjugate was washed away and 100 µl freshly prepared substrate solution (tetramethyl benzidine (TMB); 2-component substrate kit; Kirkegaard and Perry, Gaithersburg, MD) was added to each well. The reaction was allowed to proceed for 10 minutes, after which the color development was stopped by the addition of 100µl/well 1.0 M H_3PO_4. The absorbance at 450 nm was read with a reference wavelength of 650 nm ($ABS_{450/650}$), using a *vmax* plate reader (Molecular Devices, Palo Alto, CA) controlled with a Macintosh Centris 650 (Apple Computers, Cupertino, CA) and DeltaSoft software (BioMetallics, Inc, Princeton, NJ).

Four of the 25 anti-WSX receptor monoclonal antibodies activated the chimeric WSX/Rse receptor in the KIRA ELISA. The antibodies were designated: 2D7, 1G4, 1E11 and 1C11.

To determine whether the four agonist anti-WSX receptor mAbs recognized the same or different epitopes, a competitive binding ELISA was performed as described in *Kim et al. J. Immunol. Method* 156:9-17 (1992) using biotinylated mAbs (Bio-mAb). Bio-mAb were prepared using N-hydroxyl succinimide as described in *Antibodies, A Laboratory Manual* Cold Spring Harbor Laboratory, Eds. Harlow E. and D. Lane, p. 341 (1988). Microtiter wells were coated with 50μl of Goat anti-hIgG-Fc and kept overnight at 4°C, blocked with 2% BSA for 1 hr, and incubated with 25 μl/well of human WSX receptor immunoadhesin (1μg/ml) for 1 hr at room temperature. After washing, a mixture of a predetermined optimal concentration of Bio-mAb bound and a thousand-fold excess of unlabeled mAb was added into each well. Following 1hr incubation at room temperature, plates were washed and the amount of Bio-mAb was detected by the addition of HRP-streptavidin. After washing the plates, the bound enzyme was detected by the addition of the substrate o-phenylenediamine dihydrochloride (OPD), and the plates were read at 490nm with an ELISA plate reader.

The ability of the mAbs to recognize murine WSX receptor was determined in a capture ELISA. Murine WSX receptor (Fig. 21) fused to a gD tag (see above) was captured by an anti-gD (5B6) coated ELISA plate. After washing, various concentrations of biotinylated mAbs were added into each well. Biotinylated mAbs bound to murine WSX receptor-gD were detected using HRP-streptavidin as described above.

To determine whether the antibodies bound membrane-bound receptor, FACS analysis was performed using 293 cells transfected with WSX receptor. 10^5 WSX receptor-transfected 293 cells were resuspended in 100μl of PBS plus 1% fetal calf serum (FSC) and incubated with 2D7 or 1G4 hybridoma cell supernatant for 30 min on ice. After washing, cells were incubated with 100μl of FITC-goat anti-mouse IgG for 30 min at 4°C. Cells were washed twice and resuspended in 150μl of PBS plus 1% FCS and analyzed by FACscan (Becton Dickinson, Mountain View, CA). The antibodies 2D7 and 1G4 bound to membrane WSX receptor according to the FACS analysis.

The properties of agonist antibodies 2D7 and 1G4 are summarized in the following table.

TABLE 2

mAb	Isotype	epitope[a]	hWSXR[b]	mWSXR[b]	Agonist[c]
2D7	IgG1	A	+++	++	+
1G4	IgG1	B	+++	+	+

[a] These mAbs are shown to recognize different epitopes by competitive binding ELISA.

[b] These results are determined by ELISA (hWSXR is human WSX receptor and mWSXR is murine WSX receptor).

[c] The agonistic activities were determined by KIRA ELISA.

EXAMPLE 14

Human Agonist Antibodies

Single-chain Fv (scFv) fragments binding to the human WSX receptor (hWSXR) were isolated from a large human scFv library (Vaughan *et al.* *Nature Biotechnology* 14:309–314 (1996)) using antigen coated on immunotubes or biotinylated antigen in conjunction with streptavidin-coated magnetic beads (Griffiths *et al.* *EMBO J.* 13:3245–3260 (1994); and Vaughan *et al.* (1996)). Briefly, immunotubes coated overnight with 10μg/ml human WSX receptor immunoadhesin (see Example 2 above) in phosphate buffered saline (PBS) were used for three rounds of panning. The humanized antibody, huMAb4D5–8 (Carter *et al.* *Proc. Natl. Acad. Sci. USA* 89:4285–4289 (1992)) was used to counter-select for antibodies binding to the Fc of the immunoadhesin. This was done by using 1mg/ml huMAb4D5-8 in solution for the panning steps. In addition, human WSX receptor extracellular domain (cleaved from the WSX receptor immunoadhesin with Genenase (Carter *et al.* *Proteins: Structure, Function and Genetics* 6:240-248 (1989)) was biotinylated and used for three rounds of panning. Individual phage following two or three rounds of panning were characterized by antigen-binding ELISA (Tables 3 and 4).

TABLE 3

Panning with human WSX receptor immunoadhesin-coated immunotubes

Phage ELISA			# clones characterized	# BstNI fingerprints
Round	hWSXR	Fc		
2	74 / 96	0 / 96	74	11[a]
3	191 / 192	1 / 192	58	8[a]

[a] Total of 11 different clones identified.

TABLE 4

Panning with biotinylated human WSX receptor

Phage ELISA			# clones characterized	# BstNI fingerprints
Round	hWSXR	Fc		
2	8 / 96	0 / 96	8	4[a]
3	49 / 192	1 / 192	49	4[a]

[a] Total of 7 different clones identified.

Clones binding to human WSX receptor were further characterized by BstNI fingerprinting of a PCR fragment encoding the scFv. A total of 18 clones were identified: 11 from the panning using immunotubes and 7 from the panning using biotinylated antigen (there was no overlap between these groups). The DNA for all 18 clones was sequenced.

Anti-huWSXR clones obtained as described above were analyzed for agonist activity in a KIRA-ELISA assay (see above and Fig. 22) firstly as scFv phage and then as scFv. The scFv phage were PEG-precipitated (Carter *et al., Mutagenesis: A Practical Approach*, McPherson, M. ed. IRL Press, Oxford, UK, Chapter 1, pp 1-25 (1991)) and resuspended in PBS prior to screening. To prepare the scFv, DNA from the clones was transformed into 33D3 cells (a non-suppressor strain for expression of soluble protein). The cells were plated onto 2YT/2%glucose/50μg per ml of carbenicillin and incubated at 37°C overnight. A 5 ml culture (2YTG: 2YT, 2% glucose, 50μg/ml carbenicillin) was innoculated and grown at 30°C overnight. The next morning, the 5ml culture was diluted into 500ml 2YTG media and grown at 30°C until OD550≈ 0.3. Then, the media was changed from 2YTG into 2YT/50μg/ml carbenicillin/2mM IPTG and grown at 30°C for 4-5 hrs for scFv production. The culture was harvested and the cell pellet was frozen at -20°C. For purification, the cell pellet was resuspended in 10ml shockate buffer (50mM TrisHCl pH8.5, 20% sucrose, 1mM EDTA) and agitated at 4°C for 1hr. The debris was spun down and supernatant was taken to be purified on Ni NTA Superose (Qiagen) column. $MgCl_2$ was added to the supernatant to 5mM and loaded onto 0.5ml Ni NTA Superose packed into a disposable columnn. The column was then washed with 2x5ml wash buffer 1 (50mM sodium phosphate, 300mM NaCl, 25mM imidazole pH 8.0) followed by 2x5ml wash 2 buffer (50mM sodium phosphate, 300mM NaCl, 50mM imidazole pH 8.0).

The scFv was then eluted with 2.5ml elution buffer (50mM sodium phosphate, 300mM NaCl, 250mM imidazole, pH8.0). The eluted pool was buffer exchanged into PBS with a NAP5 column (Pharmacia) and stored at 4°C.

Clones #3, #4 and #17 were found to have agonist activity as phage and as scFv (see Figs. 23 and 24). The sequences of these agonist clones are shown in Fig. 25. The activity of the antibodies as $F(ab')_2$ in the KIRA ELISA was assessed, with clone #4 and clone # 17 showing enhanced activity as $F(ab')_2$. The ability of the antibodies to bind murine WSX receptor in a capture ELISA (see Example 13) was assessed. Clone #4 and clone # 17 bound murine WSX receptor in this assay.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Carter, Paul J.
Chiang, Nancy Y.
Kyung, Jin Kim
Matthews, William
Rodrigues, Maria L.

(ii) TITLE OF INVENTION: WSX RECEPTOR AGONIST ANTIBODIES

(iii) NUMBER OF SEQUENCES: 51

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: WinPatin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/667197
(B) FILING DATE: 06/20/96

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/585005
(B) FILING DATE: 01/08/96

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lee, Wendy M.
(B) REGISTRATION NUMBER: 40,378
(C) REFERENCE/DOCKET NUMBER: P0986P2

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/225-1994
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4102 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCTCGA GTCGACGGCG GGCGTTAAAG CTCTCGTGGC ATTATCCTTC 50

AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA 100

TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAAA ATTCTGTGTG 150

GTTTTGTTAC ATTGGGAATT TATTTATGTG ATAACTGCGT TTAACTTGTC 200

ATATCCAATT ACTCCTTGGA GATTTAAGTT GTCTTGCATG CCACCAAATT 250

CAACCTATGA CTACTTCCTT TTGCCTGCTG GACTCTCAAA GAATACTTCA 300

AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG 350

TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTTGCTTTC 400

GGAGTGAGCA AGATAGAAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA 450

AAGACATTTG TTTCAACAGT AAATTCTTTA GTTTTTCAAC AAATAGATGC 500

AAACTGGAAC ATACAGTGCT GGCTAAAAGG AGACTTAAAA TTATTCATCT 550

GTTATGTGGA GTCATTATTT AAGAATCTAT TCAGGAATTA TAACTATAAG 600

GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT 650

TCCCCAAAAA GGCAGTTTTC AGATGGTTCA CTGCAATTGC AGTGTTCATG 700

AATGTTGTGA ATGTCTTGTG CCTGTGCCAA CAGCCAAACT CAACGACACT 750

CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC 800

TCTAATGTCA GTTCAGCCCA TAAATATGGT GAAGCCTGAT CCACCATTAG 850

GTTTGCATAT GGAAATCACA GATGATGGTA ATTTAAAGAT TTCTTGGTCC 900

AGCCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCAGA 950

GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT 1000

CCCTGCTAGT AGACAGTATA CTTCCTGGGT CTTCGTATGA GGTTCAGGTG 1050

AGGGGCAAGA GACTGGATGG CCCAGGAATC TGGAGTGACT GGAGTACTCC 1100

TCGTGTCTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAAATTCTGA 1150

CAAGTGTTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC 1200

AAGATTGTTC CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA 1250

AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAAGTTA 1300

CTTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAAGTT TACCTATGAT 1350

GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT 1400

ATATGTGATT GATGTCAATA TCAATATCTC ATGTGAAACT GATGGGTACT 1450

TAACTAAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCACTTGCG 1500

GAAAGCACTT TGCAATTGAG GTATCATAGG AGCAGCCTTT ACTGTTCTGA 1550

TATTCCATCT ATTCATCCCA TATCTGAGCC CAAAGATTGC TATTTGCAGA 1600

GTGATGGTTT TTATGAATGC ATTTTCCAGC CAATCTTCCT ATTATCTGGC 1650

TACACAATGT GGATTAGGAT CAATCACTCT CTAGGTTCAC TTGACTCTCC 1700

ACCAACATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA 1750

GTGTGAAAGC AGAAATTACT ATAAACATTG GATTATTGAA AATATCTTGG 1800

GAAAAGCCAG TCTTTCCAGA GAATAACCTT CAATTCCAGA TTCGCTATGG 1850

TTTAAGTGGA AAAGAAGTAC AATGGAAGAT GTATGAGGTT TATGATGCAA 1900

AATCAAAATC TGTCAGTCTC CCAGTTCCAG ACTTGTGTGC AGTCTATGCT 1950

GTTCAGGTGC GCTGTAAGAG GCTAGATGGA CTGGGATATT GGAGTAATTG 2000

GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAAGTT CCTATGAGAG 2050
GACCTGAATT TTGGAGAATA ATTAATGGAG ATACTATGAA AAAGGAGAAA 2100
AATGTCACTT TACTTTGGAA GCCCCTGATG AAAAATGACT CATTGTGCAG 2150
TGTTCAGAGA TATGTGATAA ACCATCATAC TTCCTGCAAT GGAACATGGT 2200
CAGAAGATGT GGGAAATCAC ACGAAATTCA CTTTCCTGTG GACAGAGCAA 2250
GCACATACTG TTACGGTTCT GGCCATCAAT TCAATTGGTG CTTCTGTTGC 2300
AAATTTTAAT TTAACCTTTT CATGGCCTAT GAGCAAAGTA AATATCGTGC 2350
AGTCACTCAG TGCTTATCCT TTAAACAGCA GTTGTGTGAT TGTTTCCTGG 2400
ATACTATCAC CCAGTGATTA CAAGCTAATG TATTTTATTA TTGAGTGGAA 2450
AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG 2500
TTAAGAAGTA TTATATCCAT GATCATTTTA TCCCCATTGA GAAGTACCAG 2550
TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAAAC CAAAGATAAT 2600
TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT 2650
TATATGTAAT TGTGCCAGTA ATTATTTCCT CTTCCATCTT ATTGCTTGGA 2700
ACATTATTAA TATCACACCA AAGAATGAAA AAGCTATTTT GGGAAGATGT 2750
TCCGAACCCC AAGAATTGTT CCTGGGCACA AGGACTTAAT TTTCAGAAGC 2800
CAGAAACGTT TGAGCATCTT TTTATCAAGC ATACAGCATC AGTGACATGT 2850
GGTCCTCTTC TTTTGGAGCC TGAAACAATT TCAGAAGATA TCAGTGTTGA 2900
TACATCATGG AAAAATAAAG ATGAGATGAT GCCAACAACT GTGGTCTCTC 2950
TACTTTCAAC AACAGATCTT GAAAAGGGTT CTGTTTGTAT TAGTGACCAG 3000
TTCAACAGTG TTAACTTCTC TGAGGCTGAG GGTACTGAGG TAACCTATGA 3050
GGACGAAAGC CAGAGACAAC CCTTTGTTAA ATACGCCACG CTGATCAGCA 3100
ACTCTAAACC AAGTGAAACT GGTGAAGAAC AAGGGCTTAT AAATAGTTCA 3150
GTCACCAAGT GCTTCTCTAG CAAAAATTCT CCGTTGAAGG ATTCTTTCTC 3200

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe
 1               5                  10                  15

Ile Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro
                20                  25                  30

Trp Arg Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp
                35                  40                  45

Tyr Phe Leu Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser
                50                  55                  60

Asn Gly His Tyr Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser
                65                  70                  75

Gly Thr His Phe Ser Asn Leu Ser Lys Thr Thr Phe His Cys Cys
                80                  85                  90

Phe Arg Ser Glu Gln Asp Arg Asn Cys Ser Leu Cys Ala Asp Asn
                95                 100                 105

Ile Glu Gly Lys Thr Phe Val Ser Thr Val Asn Ser Leu Val Phe
               110                 115                 120

Gln Gln Ile Asp Ala Asn Trp Asn Ile Gln Cys Trp Leu Lys Gly
               125                 130                 135

Asp Leu Lys Leu Phe Ile Cys Tyr Val Glu Ser Leu Phe Lys Asn
               140                 145                 150

Leu Phe Arg Asn Tyr Asn Tyr Lys Val His Leu Leu Tyr Val Leu
               155                 160                 165

Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro Gln Lys Gly Ser
               170                 175                 180

Phe Gln Met Val His Cys Asn Cys Ser Val His Glu Cys Cys Glu
               185                 190                 195

Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr Leu Leu
               200                 205                 210

Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Gln Ser Pro
               215                 220                 225
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```
Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro
                230                 235                 240

Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile
                245                 250                 255

Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln
                260                 265                 270

Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp
                275                 280                 285

Lys Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro
                290                 295                 300

Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly
                305                 310                 315

Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr
                320                 325                 330

Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly
                335                 340                 345

Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile
                350                 355                 360

Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys
                365                 370                 375

Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys
                380                 385                 390

Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe
                395                 400                 405

Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His
                410                 415                 420

Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser
                425                 430                 435

Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
                440                 445                 450

Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg
                455                 460                 465
```

Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His
470 475 480

Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe
485 490 495

Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr
500 505 510

Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro
515 520 525

Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro
530 535 540

Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys
545 550 555

Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe
560 565 570

Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met
575 580 585

Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val
590 595 600

Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg
605 610 615

Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr
620 625 630

Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe
635 640 645

Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val
650 655 660

Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser Leu Cys Ser
665 670 675

Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn Gly Thr
680 685 690

Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu Trp
695 700 705

Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile
710 715 720

Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met
725 730 735

Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn
740 745 750

Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr
755 760 765

Lys Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp
770 775 780

Gly Glu Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr
785 790 795

Tyr Ile His Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser
800 805 810

Leu Tyr Pro Ile Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
815 820 825

Asn Ser Phe Thr Gln Asp Asp Ile Glu Lys His Gln Ser Asp Ala
830 835 840

Gly Leu Tyr Val Ile Val Pro Val Ile Ile Ser Ser Ser Ile Leu
845 850 855

Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
860 865 870

Phe Trp Glu Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln
875 880 885

Gly Leu Asn Phe Gln Lys Pro Glu Thr Phe Glu His Leu Phe Ile
890 895 900

Lys His Thr Ala Ser Val Thr Cys Gly Pro Leu Leu Leu Glu Pro
905 910 915

Glu Thr Ile Ser Glu Asp Ile Ser Val Asp Thr Ser Trp Lys Asn
920 925 930

Lys Asp Glu Met Met Pro Thr Thr Val Val Ser Leu Leu Ser Thr
935 940 945

Thr Asp Leu Glu Lys Gly Ser Val Cys Ile Ser Asp Gln Phe Asn
950 955 960

Ser Val Asn Phe Ser Glu Ala Glu Gly Thr Glu Val Thr Tyr Glu
965 970 975

Asp Glu Ser Gln Arg Gln Pro Phe Val Lys Tyr Ala Thr Leu Ile
980 985 990

Ser Asn Ser Lys Pro Ser Glu Thr Gly Glu Glu Gln Gly Leu Ile
995 1000 1005

Asn Ser Ser Val Thr Lys Cys Phe Ser Ser Lys Asn Ser Pro Leu
1010 1015 1020

Lys Asp Ser Phe Ser Asn Ser Ser Trp Glu Ile Glu Ala Gln Ala
1025 1030 1035

Phe Phe Ile Leu Ser Asp Gln His Pro Asn Ile Ile Ser Pro His
1040 1045 1050

Leu Thr Phe Ser Glu Gly Leu Asp Glu Leu Leu Lys Leu Glu Gly
1055 1060 1065

Asn Phe Pro Glu Glu Asn Asn Asp Lys Lys Ser Ile Tyr Tyr Leu
1070 1075 1080

Gly Val Thr Ser Ile Lys Lys Arg Glu Ser Gly Val Leu Leu Thr
1085 1090 1095

Asp Lys Ser Arg Val Ser Cys Pro Phe Pro Ala Pro Cys Leu Phe
1100 1105 1110

Thr Asp Ile Arg Val Leu Gln Asp Ser Cys Ser His Phe Val Glu
1115 1120 1125

Asn Asn Ile Asn Leu Gly Thr Ser Ser Lys Lys Thr Phe Ala Ser
1130 1135 1140

Tyr Met Pro Gln Phe Gln Thr Cys Ser Thr Gln Thr His Lys Ile
1145 1150 1155

Met Glu Asn Lys Met Cys Asp Leu Thr Val
1160 1165

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 896 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe
 1               5                  10                  15

Ile Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro
                20                  25                  30

Trp Arg Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp
                35                  40                  45

Tyr Phe Leu Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser
                50                  55                  60

Asn Gly His Tyr Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser
                65                  70                  75

Gly Thr His Phe Ser Asn Leu Ser Lys Thr Thr Phe His Cys Cys
                80                  85                  90

Phe Arg Ser Glu Gln Asp Arg Asn Cys Ser Leu Cys Ala Asp Asn
                95                 100                 105

Ile Glu Gly Lys Thr Phe Val Ser Thr Val Asn Ser Leu Val Phe
               110                 115                 120

Gln Gln Ile Asp Ala Asn Trp Asn Ile Gln Cys Trp Leu Lys Gly
               125                 130                 135

Asp Leu Lys Leu Phe Ile Cys Tyr Val Glu Ser Leu Phe Lys Asn
               140                 145                 150

Leu Phe Arg Asn Tyr Asn Tyr Lys Val His Leu Leu Tyr Val Leu
               155                 160                 165

Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro Gln Lys Gly Ser
               170                 175                 180

Phe Gln Met Val His Cys Asn Cys Ser Val His Glu Cys Cys Glu
               185                 190                 195
```

Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr Leu Leu
200 205 210

Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Gln Ser Pro
215 220 225

Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro
230 235 240

Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile
245 250 255

Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln
260 265 270

Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp
275 280 285

Lys Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro
290 295 300

Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly
305 310 315

Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr
320 325 330

Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly
335 340 345

Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile
350 355 360

Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys
365 370 375

Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys
380 385 390

Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe
395 400 405

Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His
410 415 420

Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser
425 430 435

Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
440 445 450

Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg
455 460 465

Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His
470 475 480

Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe
485 490 495

Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr
500 505 510

Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro
515 520 525

Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro
530 535 540

Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys
545 550 555

Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe
560 565 570

Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met
575 580 585

Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val
590 595 600

Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg
605 610 615

Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr
620 625 630

Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe
635 640 645

Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val
650 655 660

Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser Leu Cys Ser
665 670 675

```
Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn Gly Thr
                680                 685                 690

Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu Trp
                695                 700                 705

Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile
                710                 715                 720

Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met
                725                 730                 735

Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn
                740                 745                 750

Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr
                755                 760                 765

Lys Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp
                770                 775                 780

Gly Glu Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr
                785                 790                 795

Tyr Ile His Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser
                800                 805                 810

Leu Tyr Pro Ile Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
                815                 820                 825

Asn Ser Phe Thr Gln Asp Asp Ile Glu Lys His Gln Ser Asp Ala
                830                 835                 840

Gly Leu Tyr Val Ile Val Pro Val Ile Ile Ser Ser Ser Ile Leu
                845                 850                 855

Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
                860                 865                 870

Phe Trp Glu Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln
                875                 880                 885

Gly Leu Asn Phe Gln Lys Arg Thr Asp Ile Leu
                890                 895 896
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 923 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe
 1               5                  10                  15

Ile Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro
                20                  25                  30

Trp Arg Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp
                35                  40                  45

Tyr Phe Leu Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser
                50                  55                  60

Asn Gly His Tyr Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser
                65                  70                  75

Gly Thr His Phe Ser Asn Leu Ser Lys Thr Thr Phe His Cys Cys
                80                  85                  90

Phe Arg Ser Glu Gln Asp Arg Asn Cys Ser Leu Cys Ala Asp Asn
                95                 100                 105

Ile Glu Gly Lys Thr Phe Val Ser Thr Val Asn Ser Leu Val Phe
               110                 115                 120

Gln Gln Ile Asp Ala Asn Trp Asn Ile Gln Cys Trp Leu Lys Gly
               125                 130                 135

Asp Leu Lys Leu Phe Ile Cys Tyr Val Glu Ser Leu Phe Lys Asn
               140                 145                 150

Leu Phe Arg Asn Tyr Asn Tyr Lys Val His Leu Leu Tyr Val Leu
               155                 160                 165

Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro Gln Lys Gly Ser
               170                 175                 180

Phe Gln Met Val His Cys Asn Cys Ser Val His Glu Cys Cys Glu
               185                 190                 195
```

Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr Leu Leu
200 205 210

Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Gln Ser Pro
215 220 225

Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro
230 235 240

Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile
245 250 255

Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln
260 265 270

Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp
275 280 285

Lys Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro
290 295 300

Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly
305 310 315

Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr
320 325 330

Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly
335 340 345

Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile
350 355 360

Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys
365 370 375

Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys
380 385 390

Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe
395 400 405

Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His
410 415 420

Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser
425 430 435

Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser
440 445 450

Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg
455 460 465

Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His
470 475 480

Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe
485 490 495

Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr
500 505 510

Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro
515 520 525

Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro
530 535 540

Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys
545 550 555

Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe
560 565 570

Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met
575 580 585

Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val
590 595 600

Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg
605 610 615

Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr
620 625 630

Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe
635 640 645

Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val
650 655 660

Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser Leu Cys Ser
665 670 675

Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn Gly Thr
680 685 690

Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu Trp
695 700 705

Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile
710 715 720

Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met
725 730 735

Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn
740 745 750

Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr
755 760 765

Lys Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp
770 775 780

Gly Glu Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr
785 790 795

Tyr Ile His Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser
800 805 810

Leu Tyr Pro Ile Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile
815 820 825

Asn Ser Phe Thr Gln Asp Asp Ile Glu Lys His Gln Ser Asp Ala
830 835 840

Gly Leu Tyr Val Ile Val Pro Val Ile Ile Ser Ser Ser Ile Leu
845 850 855

Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu
860 865 870

Phe Trp Glu Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln
875 880 885

Gly Leu Asn Phe Gln Lys Met Phe Arg Thr Pro Arg Ile Val Pro
890 895 900

Gly His Lys Asp Leu Ile Phe Arg Arg Cys Leu Lys Ala Ala Cys
905 910 915

Ser Leu Arg Val Ile Thr Thr Pro
920 923

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3004 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCCGGG TTAAAGCTCT CGTGGCATTA TCCTTCAGTG GGGCTATTGG 50

ACTGACTTTT CTTATGCTGG GATGTGCCTT AGAGGATTAT GGATTTGCCA 100

GTTCACCCTG ACCATCTTGA AAATAAGTTA TCTCTGATCT CTGTCTGTAT 150

GTTACTTCTC TCCCCTCACC AATGGAGAAC AAATGTGGGC AAAGTGTACT 200

TCTCTGAAGT AAGATGATTT GTCAAAAATT CTGTGTGGTT TTGTTACATT 250

GGGAATTTAT TTATGTGATA ACTGCGTTTA ACTTGTCATA TCCAATTACT 300

CCTTGGAGAT TTAAGTTGTC TTGCATGCCA CCAAATTCAA CCTATGACTA 350

CTTCCTTTTG CCTGCTGGAC TCTCAAAGAA TACTTCAAAT TCGAATGGAC 400

ATTATGAGAC AGCTGTTGAA CCTAAGTTTA ATTCAAGTGG TACTCACTTT 450

TCTAACTTAT CCAAAACAAC TTTCCACTGT TGCTTTCGGA GTGAGCAAGA 500

TAGAAACTGC TCCTTATGTG CAGACAACAT TGAAGGAAAG ACATTTGTTT 550

CNACAGTAAA TTCTTTAGTT TTTCAACAAA TAGATGCAAA CTGGAACATA 600

CAGTGCTGGC TAAAAGGAGA CTTAAAATTA TTCATCTGTT ATGTGGAGTC 650

ATTATTTAAG AATCTATTCA GGAATTATAA CTATAAGGTC CATCTTTTAT 700

ATGTTCTGCC TGAAGTGTTA GAAGATTCAC CTCTGGTTCC CCAAAAAGGC 750

AGTTTTCAGA TGGTTCACTG CAATTGCAGT GTTCATGAAT GTTGTGAATG 800

TCTTGTGCCT GTGCCAACAG CCAAACTCAA CGACACTCTC CTTATGTGTT 850

TGAAAATCAC ATCTGGTGGA GTAATTTTCC AGTCACCTCT AATGTCAGTT 900

CAGCCCATAA ATATGGTGAA GCCTGATCCA CCATTAGGTT TGCATATGGA 950

AATCACAGAT GATGGTAATT TAAAGATTTC TTGGTCCAGC CCACCATTGG 1000

TACCATTTCC ACTTCAATAT CAAGTGAAAT ATTCAGAGAA TTCTACAACA 1050

GTTATCAGAG AAGCTGACAA GATTGTCTCA GCTACATCCC TGCTAGTAGA 1100

CAGTATACTT CCTGGGTCTT CGTATGAGGT TCAGGTGAGG GGCAAGAGAC 1150

TGGATGGCCC AGGAATCTGG AGTGACTGGA GTACTCCTCG TGTCTTTACC 1200

ACACAAGATG TCATATACTT TCCACCTAAA ATTCTGACAA GTGTTGGGTC 1250

TAATGTTTCT TTTCACTGCA TCTATAAGAA GGAAAACAAG ATTGTTCCCT 1300

CAAAAGAGAT TGTTTGGTGG ATGAATTTAG CTGAGAAAAT TCCTCAAAGC 1350

CAGTATGATG TTGTGAGTGA TCATGTTAGC AAAGTTACTT TTTTCAATCT 1400

GAATGAAACC AAACCTCGAG GAAAGTTTAC CTATGATGCA GTGTACTGCT 1450

GCAATGAACA TGAATGCCAT CATCGCTATG CTGAATTATA TGTGATTGAT 1500

GTCAATATCA ATATCTCATG TGAAACTGAT GGGTACTTAA CTAAAATGAC 1550

TTGCAGATGG TCAACCAGTA CAATCCAGTC ACTTGCGGAA AGCACTTTGC 1600

AATTGAGGTA TCATAGGAGC AGCCTTTACT GTTCTGATAT TCCATCTATT 1650

CATCCCATAT CTGAGCCCAA AGATTGCTAT TTGCAGAGTG ATGGTTTTTA 1700

TGAATGCATT TTCCAGCCAA TCTTCCTATT ATCTGGCTAC ACAATGTGGA 1750

TTAGGATCAA TCACTCTCTA GGTTCACTTG ACTCTCCACC AACATGTGTC 1800

CTTCCTGATT CTGTGGTGAA GCCACTGCCT CCATCCAGTG TGAAAGCAGA 1850

AATTACTATA AACATTGGAT TATTGAAAAT ATCTTGGGAA AAGCCAGTCT 1900

TTCCAGAGAA TAACCTTCAA TTCCAGATTC GCTATGGTTT AAGTGGAAAA 1950

GAAGTACAAT GGAAGATGTA TGAGGTTTAT GATGCAAAAT CAAAATCTGT 2000

CAGTCTCCCA GTTCCAGACT TGTGTGCAGT CTATGCTGTT CAGGTGCGCT 2050

GTAAGAGGCT AGATGGACTG GGATATTGGA GTAATTGGAG CAATCCAGCC 2100

TACACAGTTG TCATGGATAT AAAAGTTCCT ATGAGAGGAC CTGAATTTTG 2150

GAGAATAATT AATGGAGATA CTATGAAAAA GGAGAAAAAT GTCACTTTAC 2200

TTTGGAAGCC CCTGATGAAA AATGACTCAT TGTGCAGTGT TCAGAGATAT 2250

GTGATAAACC ATCATACTTC CTGCAATGGA ACATGGTCAG AAGATGTGGG 2300

AAATCACACG AAATTCACTT TCCTGTGGAC AGAGCAAGCA CATACTGTTA 2350

CGGTTCTGGC CATCAATTCA ATTGGTGCTT CTGTTGCAAA TTTTAATTTA 2400

ACCTTTTCAT GGCCTATGAG CAAAGTAAAT ATCGTGCAGT CACTCAGTGC 2450

TTATCCTTTA AACAGCAGTT GTGTGATTGT TTCCTGGATA CTATCACCCA 2500

GTGATTACAA GCTAATGTAT TTTATTATTG AGTGGAAAAA TCTTAATGAA 2550

GATGGTGAAA TAAAATGGCT TAGAATCTCT TCATCTGTTA AGAAGTATTA 2600

TATCCATGAT CATTTTATCC CCATTGAGAA GTACCAGTTC AGTCTTTACC 2650

CAATATTTAT GGAAGGAGTG GGAAAACCAA AGATAATTAA TAGTTTCACT 2700

CAAGATGATA TTGAAAAACA CCAGAGTGAT GCAGGTTTAT ATGTAATTGT 2750

GCCAGTAATT ATTTCCTCTT CCATCTTATT GCTTGGAACA TTATTAATAT 2800

CACACCAAAG AATGAAAAAG CTATTTTGGG AAGATGTTCC GAACCCCAAG 2850

AATTGTTCCT GGGCACAAGG ACTTAATTTT CAGAAGAGAA CGGACATTCT 2900

TTGAAGTCTA ATCATGATCA CTACAGATGA ACCCAATGTG CCAACTTCCC 2950

AACAGTCTAT AGAGTATTAG AAGATTTTTA CATTTTGAAG AAGGGCCGGA 3000

ATTC 3004

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3102 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAATTCTCGA GTCGACGGCG GGCGTTAAAG CTCTCGTGGC ATTATCCTTC 50
AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA 100
TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAAA ATTCTGTGTG 150
GTTTTGTTAC ATTGGGAATT TATTTATGTG ATAACTGCGT TTAACTTGTC 200
ATATCCAATT ACTCCTTGGA GATTTAAGTT GTCTTGCATG CCACCAAATT 250
CAACCTATGA CTACTTCCTT TTGCCTGCTG GACTCTCAAA GAATACTTCA 300
AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG 350
TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTTGCTTTC 400
GGAGTGAGCA AGATAGAAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA 450
AAGACATTTG TTTCAACAGT AAATTCTTTA GTTTTTCAAC AAATAGATGC 500
AAACTGGAAC ATACAGTGCT GGCTAAAAGG AGACTTAAAA TTATTCATCT 550
GTTATGTGGA GTCATTATTT AAGAATCTAT TCAGGAATTA TAACTATAAG 600
GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT 650
TCCCCAAAAA GGCAGTTTTC AGATGGTTCA CTGCAATTGC AGTGTTCATG 700
AATGTTGTGA ATGTCTTGTG CCTGTGCCAA CAGCCAAACT CAACGACACT 750
CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC 800
TCTAATGTCA GTTCAGCCCA TAAATATGGT GAAGCCTGAT CCACCATTAG 850
GTTTGCATAT GGAAATCACA GATGATGGTA ATTTAAAGAT TTCTTGGTCC 900
AGCCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCAGA 950
GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT 1000
CCCTGCTAGT AGACAGTATA CTTCCTGGGT CTTCGTATGA GGTTCAGGTG 1050
AGGGGCAAGA GACTGGATGG CCCAGGAATC TGGAGTGACT GGAGTACTCC 1100

TCGTGTCTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAAATTCTGA 1150

CAAGTGTTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC 1200

AAGATTGTTC CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA 1250

AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAAGTTA 1300

CTTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAAGTT TACCTATGAT 1350

GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT 1400

ATATGTGATT GATGTCAATA TCAATATCTC ATGTGAAACT GATGGGTACT 1450

TAACTAAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCACTTGCG 1500

GAAAGCACTT TGCAATTGAG GTATCATAGG AGCAGCCTTT ACTGTTCTGA 1550

TATTCCATCT ATTCATCCCA TATCTGAGCC CAAAGATTGC TATTTGCAGA 1600

GTGATGGTTT TTATGAATGC ATTTTCCAGC CAATCTTCCT ATTATCTGGC 1650

TACACAATGT GGATTAGGAT CAATCACTCT CTAGGTTCAC TTGACTCTCC 1700

ACCAACATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA 1750

GTGTGAAAGC AGAAATTACT ATAAACATTG GATTATTGAA AATATCTTGG 1800

GAAAAGCCAG TCTTTCCAGA GAATAACCTT CAATTCCAGA TTCGCTATGG 1850

TTTAAGTGGA AAAGAAGTAC AATGGAAGAT GTATGAGGTT TATGATGCAA 1900

AATCAAAATC TGTCAGTCTC CCAGTTCCAG ACTTGTGTGC AGTCTATGCT 1950

GTTCAGGTGC GCTGTAAGAG GCTAGATGGA CTGGGATATT GGAGTAATTG 2000

GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAAGTT CCTATGAGAG 2050

GACCTGAATT TTGGAGAATA ATTAATGGAG ATACTATGAA AAAGGAGAAA 2100

AATGTCACTT TACTTTGGAA GCCCCTGATG AAAAATGACT CATTGTGCAG 2150

TGTTCAGAGA TATGTGATAA ACCATCATAC TTCCTGCAAT GGAACATGGT 2200

CAGAAGATGT GGGAAATCAC ACGAAATTCA CTTTCCTGTG GACAGAGCAA 2250

GCACATACTG TTACGGTTCT GGCCATCAAT TCAATTGGTG CTTCTGTTGC 2300

AAATTTTAAT TTAACCTTTT CATGGCCTAT GAGCAAAGTA AATATCGTGC 2350

AGTCACTCAG TGCTTATCCT TTAAACAGCA GTTGTGTGAT TGTTTCCTGG 2400

ATACTATCAC CCAGTGATTA CAAGCTAATG TATTTTATTA TTGAGTGGAA 2450

AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG 2500

TTAAGAAGTA TTATATCCAT GATCATTTTA TCCCCATTGA GAAGTACCAG 2550

TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAAAC CAAAGATAAT 2600

TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT 2650

TATATGTAAT TGTGCCAGTA ATTATTTCCT CTTCCATCTT ATTGCTTGGA 2700

ACATTATTAA TATCACACCA AAGAATGAAA AAGCTATTTT GGGAAGATGT 2750

TCCGAACCCC AAGAATTGTT CCTGGGCACA AGGACTTAAT TTTCAGAAGA 2800

TGTTCCGAAC CCCAAGAATT GTTCCTGGGC ACAAGGACTT AATTTTCAGA 2850

AGATGCTTGA AGGCAGCATG TTCGTTAAGA GTCATCACCA CTCCCTAATC 2900

TCAAGTACCC AGGGACACAA ACACTGCGGA AGGCCACAGG GTCCTCTGCA 2950

TAGGAAAACC AGAGACCTTT GTTCACTTGT TTATCTGCTG ACCCTCCCTC 3000

CACTATTGTC CTATGACCCT GCCAAATCCC CCTCTGTGAG AAACACCCAA 3050

GAATGATCAA TAAAAAAAAA AAAAAAAAAA AAAAAAGTCG ACTCGAGAAT 3100

TC 3102

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 783 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe
1 5 10 15

Leu Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro
20 25 30

Trp Lys Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp
35 40 45

Ser Phe Leu Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu
50 55 60

Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser
65 70 75

Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys
80 85 90

Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn
95 100 105

Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe
110 115 120

Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly
125 130 135

Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn
140 145 150

Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu
155 160 165

Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser
170 175 180

Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys
185 190 195

His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met
200 205 210

Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu
215 220 225

Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
230 235 240

Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser
245 250 255

Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val
260 265 270

Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile
275 280 285

Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser
290 295 300

Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly
305 310 315

Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp
320 325 330

Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn
335 340 345

Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Val Ser
350 355 360

Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro
365 370 375

Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr
380 385 390

Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr
395 400 405

Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr
410 415 420

Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu
425 430 435

Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser
440 445 450

Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His
455 460 465

Arg Cys Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
470 475 480

Ser Glu Pro Lys Thr Ala Ser Tyr Arg Glu Thr Ala Phe Met Asn
485 490 495

Val Phe Ser Ser Gln Ser Phe Tyr Tyr Leu Ala Ile Gln Cys Gly
500 505 510

Phe Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr
515 520 525

Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn
530 535 540

Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser
545 550 555

Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile
560 565 570

Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu
575 580 585

Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp
590 595 600

Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp
605 610 615

Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu
620 625 630

Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg
635 640 645

Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu
650 655 660

Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg
665 670 675

Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser
680 685 690

Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu
695 700 705

Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
710 715 720

Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys
725 730 735

Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser
740 745 750

Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu
755 760 765

Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly
770 775 780

Met Lys Trp
783

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2868 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGCCCCCCC TCGAAGTCGA CGGTATCGAT AAGCTTGATA TCGAATTCCG 50

GCCGGGACAC AGGTGGGACA CTCTTTTAGT CCTCAATCCC TGGCGCGAGG 100

CCACCCAAGG CAACGCAGGA CGCAGGGCGT TTGGGGACCA GGCAGCAGAC 150

TGGGGCGGTA CCTGCGGAGA GCCACGCAAC TTCTCCAGGC CTCTGACTAC 200

TTTGGAAACT GCCCGGGGCT GCGACATCAA CCCCTTAAGT CCCGGAGGCG 250

GAAAGAGGGT GGGTTGGTTT GAAAGACACA AGGAAGAAAA ATGTGCTGTG 300

GGGCGGGTTA AGTTTCCCAC CCTCTTCCCC CTTCCCGAGC AAATTAGAAA 350

CAAAACAAAT AGAAAAGCCA GCCCTCCGGC CAACCAAAGC CCCAAGCGGA 400

GCCCCAAGCG GAGCCCCAGC CGGAGCACTC CTTTAAAAGG ATTTGCAGCG 450

GTGAGGAAAA AACCAGACCC GACCGAGGAA TCGTTCTGCA AATCCAGGTG 500

TACACCTCTG AAGAAAGATG ATGTGTCAGA AATTCTATGT GGTTTTGTTA 550

CACTGGGAAT TTCTTTATGT GATAGCTGCA CTTAACCTGG CATATCCAAT 600

CTCTCCCTGG AAATTTAAGT TGTTTTGTGG ACCACCGAAC ACAACCGATG 650

ACTCCTTTCT CTCACCTGCT GGAGCCCCAA ACAATGCCTC GGCTTTGAAG 700

GGGGCTTCTG AAGCAATTGT TGAAGCTAAA TTTAATTCAA GTGGTATCTA 750

CGTTCCTGAG TTATCCAAAA CAGTCTTCCA CTGTTGCTTT GGGAATGAGC 800

AAGGTCAAAA CTGCTCTGCA CTCACAGACA ACACTGAAGG GAAGACACTG 850

GCTTCAGTAG TGAAGGCTTC AGTTTTTCGC CAGCTAGGTG TAAACTGGGA 900

CATAGAGTGC TGGATGAAAG GGGACTTGAC ATTATTCATC TGTCATATGG 950

AGCCATTACC TAAGAACCCC TTCAAGAATT ATGACTCTAA GGTCCATCTT 1000

TTATATGATC TGCCTGAAGT CATAGATGAT TCGCCTCTGC CCCCACTGAA 1050

AGACAGCTTT CAGACTGTCC AATGCAACTG CAGTCTTCGG GGATGTGAAT 1100

GTCATGTGCC AGTACCCAGA GCCAAACTCA ACTACGCTCT TCTGATGTAT 1150

TTGGAAATCA CATCTGCCGG TGTGAGTTTT CAGTCACCTC TGATGTCACT 1200

GCAGCCCATG CTTGTTGTGA AACCCGATCC ACCCTTAGGT TTGCATATGG 1250

AAGTCACAGA TGATGGTAAT TTAAAGATTT CTTGGGACAG CCAAACAATG 1300

GCACCATTTC CGCTTCAATA TCAGGTGAAA TATTTAGAGA ATTCTACAAT 1350

TGTAAGAGAG GCTGCTGAAA TTGTCTCAGC TACATCTCTG CTGGTAGACA 1400

GTGTGCTTCC TGGATCTTCA TATGAGGTCC AGGTGAGGAG CAAGAGACTG 1450

GATGGTTCAG GAGTCTGGAG TGACTGGAGT TCACCTCAAG TCTTTACCAC 1500

ACAAGATGTT GTGTATTTTC CACCCAAAAT TCTGACTAGT GTTGGATCGA 1550

ATGCTTCCTT TCATTGCATC TACAAAAACG AAAACCAGAT TGTCTCCTCA 1600

AAACAGATAG TTTGGTGGAG GAATCTAGCT GAGAAAATCC CTGAGATACA 1650

GTACAGCATT GTGAGTGACC GAGTTAGCAA AGTTACCTTC TCCAACCTGA 1700

AAGCCACCAG ACCTCGAGGG AAGTTTACCT ATGACGCAGT GTACTGCTGC 1750

AATGAGCAGG CGTGCCATCA CCGCTATGCT GAATTATACG TGATCGATGT 1800

CAATATCAAT ATATCATGTG AAACTGACGG GTACTTAACT AAAATGACTT 1850

GCAGATGGTC ACCCAGCACA ATCCAATCAC TAGTGGGAAG CACTGTGCAG 1900

CTGAGGTATC ACAGGTGCAG CCTGTATTGT CCTGATAGTC CATCTATTCA 1950

TCCTACGTCT GAGCCCAAAA CTGCGTCTTA CAGAGAGACG GCTTTTATGA 2000

ATGTGTTTTC CAGCCAATCT TTCTATTATC TGGCTATACA ATGTGGATTC 2050

AGGATCAACC ATTCTTTAGG TTCACTTGAC TCGCCACCAA CGTGTGTCCT 2100

TCCTGACTCC GTAGTAAAAC CACTACCTCC ATCTAACGTA AAAGCAGAGA 2150

TTACTGTAAA CACTGGATTA TTGAAAGTAT CTTGGGAAAA GCCAGTCTTT 2200

CCGGAGAATA ACCTTCAATT CCAGATTCGA TATGGCTTAA GTGGAAAAGA 2250

AATACAATGG AAGACACATG AGGTATTCGA TGCAAAGTCA AAGTCTGCCA 2300

GCCTGCTGGT GTCAGACCTC TGTGCAGTCT ATGTGGTCCA GGTTCGCTGC 2350

CGGCGGTTGG ATGGACTAGG ATATTGGAGT AATTGGAGCA GTCCAGCCTA 2400

TACGCTTGTC ATGGATGTAA AAGTTCCTAT GAGAGGGCCT GAATTTTGGA 2450

GAAAAATGGA TGGGGACGTT ACTAAAAAGG AGAGAAATGT CACCTTGCTT 2500

TGGAAGCCCC TGACGAAAAA TGACTCACTG TGTAGTGTGA GGAGGTACGT 2550

GGTGAAGCAT CGTACTGCCC ACAATGGGAC GTGGTCAGAA GATGTGGGAA 2600

ATCGGACCAA TCTCACTTTC CTGTGGACAG AACCAGCGCA CACTGTTACA 2650

GTTCTGGCTG TCAATTCCCT CGGCGCTTCC CTTGTGAATT TTAACCTTAC 2700

CTTCTCATGG CCCATGAGTA AAGTGAGTGC TGTGGAGTCA CTCAGTGCTT 2750

ATCCCCTGAG CAGCAGCTGT GTCATCCTTT CCTGGACACT GTCACCTGAT 2800

GATTATAGTC TGTTATATCT GGTTATTGAA TGGAAGATCC TTAATGAAGA 2850

TGATGGAATG AAGTGGCT 2868

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGTTAAGTT TCCCACCC 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGTGGGAAA CTTAACCC 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGGATACAGT GGGATCCC 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCCGAGCAC TCCTTTAA 18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTAAAGGAGT GCTCCCGC 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GAGCGGCCCT GTTAGATA 18

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTATACACCT CTGAAGAA 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTCTTCAGAG GTGTACAC 18

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGCGAGGCT ACTTCTAT 18

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCCCTGG AAATTTAA 18

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTAAATTTCC AGGGAGAG 18

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATTTGAAGGA GTTAAGCC 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AATTTAATTC AAGTGGTA 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TACCAGTTGA ATTAAATT 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTATCACTTC ATAATATA 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GATGGTCAGG GTGAACTG 18

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CAGTTCACCC TGACCATC 18

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GAGGCGAATG TGCGGATT 18

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTAAATCTC CAAGGAGT 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ACTCCTTGGA GATTTAAG 18

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAGTCTTAAG CCAGACTT 18

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTAAGGCAC ATCCCAGC 18

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCTGGGATGT GCCTTAGA 18

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGCAATGAAT TGACCCCC 18

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TACTTCAGAG AAGTACAC 18

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTGTACTTCT CTGAAGTA 18

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAATCACGGT AACTATCA 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CAGCTGTCTC ATAATGTC 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACATTATGA GACAGCTG 18

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TTCGTCAAGC CATCTGAT 18

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

His Gln Asn Leu Ser Asp Gly Lys
1 5 8

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

His Gln Asn Ile Ser Asp Gly Lys
1 5 8

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

```
His Gln Ser Leu Gly Thr Gln
  1                 5     7
```

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```
Val Ile Ser Ser His Leu Gly Gln
  1                 5         8
```

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

```
Pro Lys Asn Ser Ser Met Ile Ser Asn Thr Pro
  1                 5                10  11
```

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
Asp Lys Thr His Thr Cys Pro Pro Cys Pro
  1                 5                10
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG 50

C 51

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7127 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TATCGATATG CATTGGGGAA CCCTGTGCGG ATTCTTGTGG 950

CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT GTGCCCATCC AAAAAGTCCA 1000

AGATGACACC AAAACCCTCA TCAAGACAAT TGTCACCAGG ATCAATGACA 1050

TTTCACACAC GCAGTCAGTC TCCTCCAAAC AGAAAGTCAC CGGTTTGGAC 1100

TTCATTCCTG GGCTCCACCC CATCCTGACC TTATCCAAGA TGGACCAGAC 1150

ACTGGCAGTC TACCAACAGA TCCTCACCAG TATGCCTTCC AGAAACGTGA 1200

TCCAAATATC CAACGACCTG GAGAACCTCC GGGATCTTCT TCACGTGCTG 1250

GCCTTCTCTA AGAGCTGCCA CTTGCCCTGG GCCAGTGGCC TGGAGACCTT 1300

GGACAGCCTG GGGGGTGTCC TGGAAGCTTC AGGCTACTCC ACAGAGGTGG 1350

TGGCCCTGAG CAGGCTGCAG GGGTCTCTGC AGGACATGCT GTGGCAGCTG 1400

GACCTCAGCC CTGGGTGCGG GGTCACCGAC AAAACTCACA CATGCCCACC 1450

GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC 1500

CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC 1550

GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA 1600

CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC 1650

AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG 1700

GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT 1750

CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG 1800

AACCACAGGT GTACACCCTG CCCCCATCCC GGGAAGAGAT GACCAAGAAC 1850

CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC 1900

CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC 1950

CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC 2000

GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT 2050

GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC 2100

CGGGTAAATG AGTGCGACGG CCCTAGAGTC GACCTGCAGA AGCTTCTAGA 2150

GTCGACCTGC AGAAGCTTGG CCGCCATGGC CCAACTTGTT TATTGCAGCT 2200

TATAATGGTT ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC 2250

ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT 2300

CTTATCATGT CTGGATCGAT CGGGAATTAA TTCGGCGCAG CACCATGGCC 2350

TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA 2400

AGAACCAGCT GTGGAATGTG TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC 2450

TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC 2500

CAGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA 2550

TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC 2600

CCGCCCCTAA CTCCGCCCAG TTCCGCCCAT TCTCCGCCCC ATGGCTGACT 2650

AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT 2700

TCCAGAAGTA GTGAGGAGGC TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG 2750

CTGTTAATTC GAACACGCAG ATGCAGTCGG GGCGGCGCGG TCCCAGGTCC 2800

ACTTCGCATA TTAAGGTGAC GCGTGTGGCC TCGAACACCG AGCGACCCTG 2850

CAGCGACCCG CTTAACAGCG TCAACAGCGT GCCGCAGATC TGATCAAGAG 2900

ACAGGATGAG GATCGTTTCG CATGATTGAA CAAGATGGAT TGCACGCAGG 2950

TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC 3000

AGACAATCGG CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG 3050

CGCCCGGTTC TTTTTGTCAA GACCGACCTG TCCGGTGCCC TGAATGAACT 3100

GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GGCGTTCCTT 3150

GCGCAGCTGT GCTCGACGTT GTCACTGAAG CGGGAAGGGA CTGGCTGCTA 3200

TTGGGCGAAG TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC 3250

CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACGCTTG 3300

ATCCGGCTAC CTGCCCATTC GACCACCAAG CGAAACATCG CATCGAGCGA 3350

GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA 3400

AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG CTCAAGGCGC 3450

GCATGCCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG 3500

CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG 3550

CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG 3600

ATATTGCTGA AGAGCTTGGC GGCGAATGGG CTGACCGCTT CCTCGTGCTT 3650

TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT 3700

TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC 3750

GACGCCCAAC CTGCCATCAC GAGATTTCGA TTCCACCGCC GCCTTCTATG 3800

AAAGGTTGGG CTTCGGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC 3850

CAGCGCGGGG ATCTCATGCT GGAGTTCTTC GCCCACCCCG GGAGATGGGG 3900

GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGGA ACCCGCGCTA 3950

TGACGGCAAT AAAAAGACAG AATAAAACGC ACGGGTGTTG GGTCGTTTGT 4000

TCATAAACGC GGGGTTCGGT CCCAGGGCTG GCACTCTGTC GATACCCCAC 4050

CGAGACCCCA TTGGGGCCAA TACGCCCGCG TTTCTTCCTT TTCCCCACCC 4100

CAACCCCCAA GTTCGGGTGA AGGCCCAGGG CTCGCAGCCA ACGTCGGGGC 4150

GGCAAGCCCG CCATAGCCAC GGGCCCCGTG GGTTAGGGAC GGGGTCCCCC 4200
ATGGGGAATG GTTTATGGTT CGTGGGGGTT ATTCTTTTGG GCGTTGCGTG 4250
GGGTCAGGTC CACGACTGGA CTGAGCAGAC AGACCCATGG TTTTTGGATG 4300
GCCTGGGCAT GGACCGCATG TACTGGCGCG ACACGAACAC CGGGCGTCTG 4350
TGGCTGCCAA ACACCCCCGA CCCCCAAAAA CCACCGCGCG GATTTCTGGC 4400
GCCGCCGGAC GAACTAAACC TGACTACGGC ATCTCTGCCC CTTCTTCGCT 4450
GGTACGAGGA GCGCTTTTGT TTTGTATTGG TCACCACGGC CGAGTTTCCG 4500
CGGGACCCCG GCCAGGGCAC CTGTCCTACG AGTTGCATGA TAAAGAAGAC 4550
AGTCATAAGT GCGGCGACGA TAGTCATGCC CCGCGCCCAC CGGAAGGAGC 4600
TGACTGGGTT GAAGGCTCTC AAGGGCATCG GTCGAGCGGC CGCATCAAAG 4650
CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT 4700
GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC 4750
CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT 4800
CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG 4850
GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC 4900
CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC 4950
TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC 5000
GGGCTATTCT TTTGATTTAT AAGGGATTTT GCCGATTTCG GCCTATTGGT 5050
TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA 5100
TTAACGTTTA CAATTTTATG GTGCAGGCCT CGTGATACGC CTATTTTTAT 5150
AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT 5200
CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC 5250
AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT 5300
ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT 5350

CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT 5400

GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA 5450

TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA 5500

GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT 5550

ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT 5600

ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT 5650

ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG 5700

TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG 5750

AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT 5800

CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC 5850

CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG 5900

AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 5950

GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 6000

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6050

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6100

ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT 6150

AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT 6200

ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG 6250

GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT 6300

TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT 6350

GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA 6400

CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT 6450

TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC 6500

TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT 6550

ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA 6600

TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG 6650

CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG 6700

CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6750

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6800

GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6850

TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT 6900

TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCTGGC 6950

ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT 7000

GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC 7050

GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA 7100

ACAGCTATGA CCATGATTAC GAATTAA 7127

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 397 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Met His Trp Gly Thr Leu Cys Gly Phe Leu Trp Leu Trp Pro Tyr
1 5 10 15

Leu Phe Tyr Val Gln Ala Val Pro Ile Gln Lys Val Gln Asp Asp
20 25 30

Thr Lys Thr Leu Ile Lys Thr Ile Val Thr Arg Ile Asn Asp Ile
35 40 45

Ser His Thr Gln Ser Val Ser Ser Lys Gln Lys Val Thr Gly Leu
50 55 60

Asp Phe Ile Pro Gly Leu His Pro Ile Leu Thr Leu Ser Lys Met
65 70 75

Asp Gln Thr Leu Ala Val Tyr Gln Gln Ile Leu Thr Ser Met Pro
80 85 90

Ser Arg Asn Val Ile Gln Ile Ser Asn Asp Leu Glu Asn Leu Arg
95 100 105

Asp Leu Leu His Val Leu Ala Phe Ser Lys Ser Cys His Leu Pro
110 115 120

Trp Ala Ser Gly Leu Glu Thr Leu Asp Ser Leu Gly Gly Val Leu
125 130 135

Glu Ala Ser Gly Tyr Ser Thr Glu Val Val Ala Leu Ser Arg Leu
140 145 150

Gln Gly Ser Leu Gln Asp Met Leu Trp Gln Leu Asp Leu Ser Pro
155 160 165

Gly Cys Gly Val Thr Asp Lys Thr His Thr Cys Pro Pro Cys Pro
170 175 180

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
185 190 195

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
200 205 210

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
215 220 225

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
230 235 240

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
245 250 255

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
260 265 270

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
275 280 285

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
290 295 300

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
305 310 315

```
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                320                 325                 330

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                335                 340                 345

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                350                 355                 360

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
                365                 370                 375

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
                380                 385                 390

Leu Ser Leu Ser Pro Gly Lys
                395     397
```

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 249 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
  1               5                  10                  15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
                 20                  25                  30

Gly Tyr Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
                 35                  40                  45

Glu Trp Met Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr
                 50                  55                  60

Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
                 65                  70                  75

Ile Gly Thr Ala Tyr Met Glu Leu Ser Arg Leu Ser Ser Asp Asp
                 80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Tyr Tyr Gly Ser Ser
                 95                 100                 105
```

Ala Tyr His Arg Gly Ser Tyr Tyr Met Asp Val Trp Gly Arg Gly
110 115 120

Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Thr Gly Gly Gly
125 130 135

Gly Ser Gly Gly Gly Gly Ser Ser Glu Leu Thr Gln Asp Pro Ala
140 145 150

Val Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly
155 160 165

Asp Ser Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro
170 175 180

Gly Gln Ala Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro
185 190 195

Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr
200 205 210

Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp
215 220 225

Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His Val Val Phe
230 235 240

Gly Gly Gly Thr Lys Leu Thr Val Leu
245 249

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 250 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

Glu Ser Leu Lys Ile Ser Cys Gln Gly Ser Gly Phe Thr Phe Ser
20 25 30

Ser Tyr Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
35 40 45

Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr
50 55 60

Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser
65 70 75

Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Val Val Val Pro Ala
95 100 105

Thr Ser Leu Arg Gly Gly Met Asp Val Trp Gly Gln Gly Thr Thr
110 115 120

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
125 130 135

Gly Gly Gly Gly Ser Gln Ser Val Leu Thr Gln Pro Ala Ser Val
140 145 150

Ser Gly Ser Pro Gly Gln Ser Ile Thr Ile Ser Cys Thr Gly Thr
155 160 165

Ser Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser Trp Tyr Gln Gln
170 175 180

His Pro Gly Lys Ala Pro Lys Leu Met Ile Tyr Glu Gly Ser Lys
185 190 195

Arg Pro Ser Gly Val Ser Asn Arg Phe Ser Gly Ser Lys Ser Gly
200 205 210

Ser Thr Ala Ser Leu Thr Ile Ser Gly Leu Gln Ala Glu Asp Glu
215 220 225

Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Thr Arg Ser Thr Arg Val
230 235 240

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
245 250

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
Gln Val Arg Leu Gln Gln Ser Gly Gly Gly Leu Val Gln Pro Gly
 1               5                  10                  15

Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
                20                  25                  30

Asp Tyr Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
                35                  40                  45

Glu Trp Val Ser Gly Met Thr Trp Asn Ser Gly Ser Ile Gly Tyr
                50                  55                  60

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
                65                  70                  75

Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Arg Glu Pro His Asn Thr Asp Ala
                95                 100                 105

Phe Asp Ile Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Gly
               110                 115                 120

Gly Gly Gly Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp
               125                 130                 135

Val Val Met Thr Gln Ser Pro Ser Phe Leu Ser Ala Phe Val Gly
               140                 145                 150

Asp Thr Ile Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Tyr Asn
               155                 160                 165

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
               170                 175                 180

Leu Ile Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg
               185                 190                 195
```

Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
200 205 210

Ser Leu Gln Pro Glu Asp Phe Gly Thr Tyr Tyr Cys Gln Gln Leu
215 220 225

Ile Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile
230 235 240

Lys
241

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 amino acids
(B) TYPE: Amino Acid
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe
1 5 10 15

Leu Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro
20 25 30

Trp Lys Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp
35 40 45

Ser Phe Leu Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu
50 55 60

Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser
65 70 75

Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys
80 85 90

Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn
95 100 105

Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe
110 115 120

Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly
125 130 135

Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn
140 145 150

Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu
155 160 165

Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser
170 175 180

Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys
185 190 195

His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met
200 205 210

Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu
215 220 225

Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
230 235 240

Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser
245 250 255

Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val
260 265 270

Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile
275 280 285

Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser
290 295 300

Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly
305 310 315

Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp
320 325 330

Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn
335 340 345

Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Ile Ser
350 355 360

Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro
365 370 375

Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr
380 385 390

Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr
395 400 405

Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr
410 415 420

Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu
425 430 435

Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser
440 445 450

Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His
455 460 465

Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr
470 475 480

Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu
485 490 495

Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp
500 505 510

Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr
515 520 525

Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn
530 535 540

Val Lys Ala Glu Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser
545 550 555

Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile
560 565 570

Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu
575 580 585

Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp
590 595 600

Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp
605 610 615

Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu
620 625 630

Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg
635 640 645

Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu
650 655 660

Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg
665 670 675

Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser
680 685 690

Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu
695 700 705

Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
710 715 720

Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys
725 730 735

Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser
740 745 750

Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu
755 760 765

Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly
770 775 780

Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile
785 790 795

His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr
800 805 810

Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly
815 820 825

Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu
830 835 840

Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu
845 850 855

Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp
860 865 870

Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu
875 880 885

Asn Phe Gln Lys Arg Thr Asp Thr Leu
890 894

WHAT IS CLAIMED IS:

1. An agonist antibody which specifically binds to the WSX receptor.

2. The antibody of claim 1 which specifically binds to human WSX receptor.

3. The antibody of claim 2 which specifically binds to human WSX receptor variant 13.2.

4. The antibody of claim 1 which binds WSX receptor with a Kd of no more than about 1×10^{-8}M.

5. The antibody of claim 4 which binds WSX receptor with a Kd of no more than about 1×10^{-9}M.

6. The antibody of claim 2 which also binds to murine WSX receptor.

7. The antibody of claim 1 which has an IC50 in a KIRA ELISA of about 0.5μg/ml or less.

8. The antibody of claim 7 which has an IC50 in a KIRA ELISA of about 0.2μg/ml or less.

9. The antibody of claim 8 which has an IC50 in a KIRA ELISA of about 0.1μg/ml or less.

10. The antibody of claim 1 which has biological characteristics of antibody 2D7 (ATCC Accession Number______).

11. The antibody of claim 10 which binds to the epitope on WSX receptor bound by antibody 2D7.

12. The antibody of claim 10 which has complementarity determining region (CDR) residues from antibody 2D7.

13. The antibody of claim 1 which has the biological characteristics of antibody 1G4 (ATCC Accession Number______).

14. The antibody of claim 13 which binds to the epitope on WSX receptor bound by antibody 1G4.

15. The antibody of claim 13 which has complementarity determining region (CDR) residues from antibody 1G4.

16. The antibody of claim 1 which has the biological characteristics of antibody 1E11 (ATCC Accession Number______).

17. The antibody of claim 16 which binds to the epitope on WSX receptor bound by antibody 1E11.

09/543230

18. The antibody of claim 16 which has complementarity determining region (CDR) residues from antibody 1E11.

19. The antibody of claim 1 which has the biological characteristics of antibody 1C11 (ATCC Accession Number______).

20. The antibody of claim 19 which binds to the epitope on WSX receptor bound by antibody 1C11.

21. The antibody of claim 19 which has complementarity determining region (CDR) residues from antibody 1C11.

22. The antibody of claim 1 comprising hypervariable region residues of clone 3 antibody (SEQ ID NO:48).

23. The antibody of claim 1 comprising hypervariable region residues of clone 4 antibody (SEQ ID NO:49).

24. The antibody of claim 1 comprising hypervariable region residues of clone 17 antibody (SEQ ID NO:50).

25. The antibody of claim 1 which is a monoclonal antibody.

26. The antibody of claim 1 which is a human antibody.

27. The antibody of claim 1 which is a humanized antibody.

28. The antibody of claim 1 which is an antibody fragment.

29. The antibody fragment of claim 28 which is an $F(ab')_2$.

30. A composition comprising the antibody of claim 1 and a physiologically acceptable carrier.

31. The composition of claim 30 which is sterile.

32. The composition of claim 31 which is lyophilized.

33. The composition of claim 30 further comprising a cytokine.

34. A method for activating the WSX receptor comprising exposing the WSX receptor to an amount of the antibody of claim 1 which is effective for activating the WSX receptor.

35. A method for enhancing proliferation or differentiation of a cell comprising the WSX receptor comprising exposing the cell to an amount of the antibody of claim 1 which is effective for enhancing proliferation or differentiation of the cell.

36. An isolated nucleic acid molecule encoding the antibody of claim 1.

37. A vector comprising the nucleic acid molecule of claim 36.

38. A host cell comprising the nucleic acid molecule of claim 36.

39. A method of producing an agonist antibody which specifically binds to the WSX receptor comprising culturing the host cell of claim 38 and recovering the antibody from the cell culture.

WSX RECEPTOR AGONIST ANTIBODIES

ABSTRACT OF THE DISCLOSURE

Agonist antibodies which bind to and activate the WSX receptor are described along with various uses for these antibodies. Preferred antibodies are those which display an IC50 in the KIRA ELISA bioassay of about 0.5μg/ml or less.

OIPE
MAY 7 1997
PATENT & TRADEMARK OFFICE

Docket No. P0986P2

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY FOR CONTINUING APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

WSX RECEPTOR AGONIST ANTIBODIES

the specification of which (check one) __ is attached hereto or X was filed on 07-Jan-1997 as Application Serial No. 08/779,457 was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate have a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):

Number	Country	Day/Month/Year Filed	Priority Claimed Yes	Priority Claimed No

---

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below:

Application Ser. No.	Filing Date
60/	08-Jan-1996

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Ser. No.	Filing Date	Status: Patented, Pending, Abandoned
08/667,197	20-June-1996	Pending
08/780,562	08-January-1997	Pending

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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I hereby declare that all statements made herein of my own knowledge and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from his foreign patent agent as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

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Citizenship (MR)
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```
sites: std
length: 4102 (circular)

           pleI
           hinfI
        xhoI   salI
        paeR7I taqI              aluI
   ecoRI taqI hincII/hindII tru9I            eco57I                                         mnlI
   apoI avaI  accI    aciI  mseI            bslI                                    fokI    ddeI
  1 GAATTCTCGA GTCGACGGCG GGCGTTAAAG CTCTCGTGGC ATTATCCTTC AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA
    CTTAAGAGCT CAGCTGCCGC CCGCAATTTC GAGAGCACCG TAATAGGAAG TCACCCCGAT AACCTGACTG AAAAGAATAC GACCCTACAC GGAATCTCCT

                                                                                              tru9I
           rsaI                                                                               mseI
           csp6I    eco57I              apoI              maeIII    apoI
101 TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAAA ATTCTGTGTG GTTTTGTTAC ATTGGGAATT TATTTATGTG ATAACTGCGT TTAACTTGTC
    AATACCCACA TGAAGAGACT TCATTCTACT AAACAGTTTT TAAGACACAC CAAAACAATG TAACCCTTAA ATAAATACAC TATTGACGCA AATTGAACAG
  1                           M  I   C  Q  K   F  C  V   V  L  L  H   W  E  F   I  Y  V   I  T  A  F   N  L  S

                                      nlaIII
                                      sphI
             styI      tru9I          nspI                                                 pleI
    xcmI     bsaJI     mseI           nspHI      apoI                                      hinfI        apoI
201 ATATCCAATT ACTCCTTGGA GATTTAAGTT GTCTTGCATG CCACCAAATT CAACCTATGA CTACTTCCTT TTGCCTGCTG GACTCTCAAA GAATACTTCA
    TATAGGTTAA TGAGGAACCT CTAAATTCAA CAGAACGTAC GGTGGTTTAA GTTGGATACT GATGAAGGAA AACGGACGAC CTGAGAGTTT CTTATGAAGT
 26 Y  P  I   T  P  W  R   F  K  L   S  C  M   P  P  N  S   T  Y  D   Y  F  L   L  P  A  G   L  S  K   N  T  S

      taqI
     sfuI
     bstBI               pvuII
     bsiCI               nspBII                 tru9I        rsaI
     asuII          bsmAI aluI         ddeI     mseI         csp6I
301 AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTTGCTTTC
    TTAAGCTTAC CTGTAATACT CTGTCGACAA CTTGGATTCA AATTAAGTTC ACCATGAGTG AAAAGATTGA ATAGGTTTTG TTGAAAGGTG ACAACGAAAG
 59 N  S  N  G   H  Y  E   T  A  V   E  P  K  F   N  S  S   G  T  H   F  S  N  L   S  K  T   T  F  H   C  C  F  R

                                                                          apoI                    sfaNI
401 GGAGTGAGCA AGATAGAAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA AAGACATTTG TTTCAACAGT AAATTCTTTA GTTTTTCAAC AAATAGATGC
    CCTCACTCGT TCTATCTTTG ACGAGGAATA CACGTCTGTT GTAACTTCCT TTCTGTAAAC AAAGTTGTCA TTTAAGAAAT CAAAAAGTTG TTTATCTACG
 93  S  E  Q   D  R  N   C  S  L  C   A  D  N   I  E  G   K  T  F  V   S  T  V   N  S  L   V  F  Q  Q   I  D  A

                                                                            xmnI
                                                                            tfiI
                                                                            hinfI
                                   tru9I                        pleI    tru9I                              sau96I
                                                                                                           avaII
     bsrI                     bsmAI mseI                        hinfI   mseI asp700                        asuI
501 AAACTGGAAC ATACAGTGCT GGCTAAAAGG AGACTTAAAA TTATTCATCT GTTATGTGGA GTCATTATTT AAGAATCTAT TCAGGAATTA TAACTATAAG
    TTTGACCTTG TATGTCACGA CCGATTTTCC TCTGAATTTT AATAAGTAGA CAATACACCT CAGTAATAAA TTCTTAGATA AGTCCTTAAT ATTGATATTC
126 N  W  N   I  Q  C  W   L  K  G   D  L  K   L  F  I  C   Y  V  E   S  L  F   K  N  L  F   R  N  Y   N  Y  K
```

Figure 1A

```
                                        tfiI                                                        nlaIII
                                        hinfI mnlI      bslI                                       rcaI
                           eco57I    mboII hphI    nlaIV                               munI        bspHI
601  GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT TCCCCAAAAA GGCAGTTTTC AGATGGTTCA CTGCAATTGC AGTGTTCATG
     CAGGTAGAAA ATATACAAGA CGGACTTCAC AATCTTCTAA GTGGAGACCA AGGGGTTTTT CCGTCAAAAG TCTACCAAGT GACGTTAACG TCACAAGTAC
159  V  H  L  L   Y  V  L   P  E  V   L  E  D  S   P  L  V   P  Q  K   G  S  F  Q   M  V  H   C  N  C   S  V  H  E

                                                                                                   hphI
                                                                                                  maeIII
                                                                                                bsrI  mnlI
701  AATGTTGTGA ATGTCTTGTG CCTGTGCCAA CAGCCAAACT CAACGACACT CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC
     TTACAACACT TACAGAACAC GGACACGGTT GTCGGTTTGA GTTGCTGTGA GAGGAATACA CAAACTTTTA GTGTAGACCA CCTCATTAAA AGGTCAGTGG
193    C  C  E   C  L  V   P  V  P  T   A  K  L   N  D  T   L  L  M  C   L  K  I   T  S  G   G  V  I  F   Q  S  P

                                             bslI
                                         sau3AI
                                         mboI/ndeII[dam-]                                             xcmI
                                         dpnI[dam+]                                   tru9I       sau96I
                                         dpnII[dam-]                                  mseI        avaII
                      bstXI    hphI      alwI[dam-]         ndeI                     ahaIII/draI  asuI
801  TCTAATGTCA GTTCAGCCCA TAAATATGGT GAAGCCTGAT CCACCATTAG GTTTGCATAT GGAAATCACA GATGATGGTA ATTTAAAGAT TTCTTGGTCC
     AGATTACAGT CAAGTCGGGT ATTTATACCA CTTCGGACTA GGTGGTAATC CAAACGTATA CCTTTAGTGT CTACTACCAT TAAATTTCTA AAGAACCAGG
226  L  M  S   V  Q  P  I   N  M  V   K  P  D   P  P  L  G   L  H  M   E  I  T   D  D  G  N   L  K  I   S  W  S

                 rsaI
                 csp6I
                nlaIV
                kpnI
                hgiCI
                banI
                asp718                                 ecoRI                      drdI      ddeI
       bslI     acc65I                       sspI      apoI                    aluI        bsmAI aluI fokI
901  AGCCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCAGA GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT
     TCGGGTGGTA ACCATGGTAA AGGTGAAGTT ATAGTTCACT TTATAAGTCT CTTAAGATGT TGTCAATAGT CTCTTCGACT GTTCTAACAG AGTCGATGTA
259  S  P  P  L   V  P  F   P  L  Q   Y  Q  V  K   Y  S  E   N  S  T   T  V  I  R   E  A  D   K  I  V   S  A  T  S
                                                             ^begin12u

                                                                                tfiI
                                                                            scrFI
                                                                            mvaI
                                                                            ecoRII
                                bpuAI                                       dsaV
                            scrFI                                      bstXI
                            mvaI bbsI                                   bstNI
                            ecoRII                                 apyI[dcm+]
                            dsaV                               sau96I              rsaI
             accI           bstNI                                   haeIII/palI         bsrI csp6I
          rmaI      bst1107I bsaJI mboII        mnlI       bsrI   asuI   hinfI     maeIII  scaI
          maeI      accI    apyI[dcm+]     mnlI    hphI        bsmAI fokI   bsaJI   gsuI/bpmI gsuI/bpmI mnlI
1001 CCCTGCTAGT AGACAGTATA CTTCCTGGGT CTTCGTATGA GGTTCAGGTG AGGGGCAAGA GACTGGATGG CCCAGGAATC TGGAGTGACT GGAGTACTCC
     GGGACGATCA TCTGTCATAT GAAGGACCCA GAAGCATACT CCAAGTCCAC TCCCCGTTCT CTGACCTACC GGGTCCTTAG ACCTCACTGA CCTCATGAGG
293    L  L  V   D  S  I   L  P  G  S   S  Y  E   V  Q  V   R  G  K  R   L  D  G   P  G  I   W  S  D  W   S  T  P
```

Figure 1B

08/779457

```
                                                                                             sfaNI
                                                  apoI
1101 TCGTGTCTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAAATTCTGA CAAGTGTTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC
     AGCACAGAAA TGGTGTGTTC TACAGTATAT GAAAGGTGGA TTTTAAGACT GTTCACAACC CAGATTACAA AGAAAAGTGA CGTAGATATT CTTCCTTTTG
 326  R  V  F   T  T  Q  D   V  I  Y   F  P  P   K  I  L  T   S  V  G   S  N  V   S  F  H  C   I  Y  K   K  E  N

                                                                                                 nlaIII
                                                                                              sau3AI
                                                                                              mboI/ndeII[dam-]
                                                                                              dpnI[dam+]
                      *                    apoI     ddeI                                      dpnII[dam-]
                mnlI                   fokI       aluI       apoI  mnlI     bsrI                 bclI[dam-]      maeIII
1201 AAGATTGTTC CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAAGTTA
     TTCTAACAAG GGAGTTTTCT CTAACAAACC ACCTACTTAA ATCGACTCTT TTAAGGAGTT TCGGTCATAC TACAACACTC ACTAGTACAA TCGTTTCAAT
 359 K  I  V  P   S  K  E   I  V  W   W  M  N  L   A  E  K   I  P  Q   S  Q  Y  D   V  V  S   D  H  V   S  K  V  T

                                   taqI
                                  xhoI
                                  paeR7I
                                  avaI
                                 mnlI mnlI               sfaNI    rsaI  fnu4HI        bsmI
                                                                  csp6I bbvI        nlaIII
1301 CTTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAAGTT TACCTATGAT GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT
     GAAAAAAGTT AGACTTACTT TGGTTTGGAG CTCCTTTCAA ATGGATACTA CGTCACATGA CGACGTTACT TGTACTTACG GTAGTAGCGA TACGACTTAA
 393  F  F  N   L  N  E   T  K  P  R   G  K  F   T  Y  D   A  V  Y  C   C  N  E   H  E  C   H  H  R  Y   A  E  L

                                                          tru9I                                  rsaI
                                                          mseI                                   csp6I
                                                      rsaI                                   bsrI          maeIII
                                    nlaIII            csp6I                          hincII/hindII bsrI            aciI
1401 ATATGTGATT GATGTCAATA TCAATATCTC ATGTGAAACT GATGGGTACT TAACTAAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCACTTGCG
     TATACACTAA CTACAGTTAT AGTTATAGAG TACACTTTGA CTACCCATGA ATTGATTTTA CTGAACGTCT ACCAGTTGGT CATGTTAGGT CAGTGAACGC
 426  Y  V  I   D  V  N  I   N  I  S   C  E  T   D  G  Y  L   T  K  M   T  C  R   W  S  T  S   T  I  Q   S  L  A

                                                                                       hgiJII
                                                                                       bsp1286
                                                                                       bmyI
                                       fnu4HI                                          banII
                  munI mnlI            bbvI                               fokI       ddeI
1501 GAAAGCACTT TGCAATTGAG GTATCATAGG AGCAGCCTTT ACTGTTCTGA TATTCCATCT ATTCATCCCA TATCTGAGCC CAAAGATTGC TATTTGCAGA
     CTTTCGTGAA ACGTTAACTC CATAGTATCC TCGTCGGAAA TGACAAGACT ATAAGGTAGA TAAGTAGGGT ATAGACTCGG GTTTCTAACG ATAAACGTCT
 459 E  S  T  L   Q  L  R   Y  H  R   S  S  L  Y   C  S  D   I  P  S   I  H  P  I   S  E  P   K  D  C   Y  L  Q  S

                                                                              sau3AI
                                                                              mboI/ndeII[dam-]
                                                                              dpnI[dam+]
                                                                              dpnII[dam-]
                                                                             alwI[dam-]
                      ppu10I                                            mamI[dam-]           rmaI         pleI
                      nsiI/avaIII
                    bsmI                mboII                draIII     bsaBI[dam-]          maeI         hinfI
1601 GTGATGGTTT TTATGAATGC ATTTTCCAGC CAATCTTCCT ATTATCTGGC TACACAATGT GGATTAGGAT CAATCACTCT CTAGGTTCAC TTGACTCTCC
     CACTACCAAA AATACTTACG TAAAAGGTCG GTTAGAAGGA TAATAGACCG ATGTGTTACA CCTAATCCTA GTTAGTGAGA GATCCAAGTG AACTGAGAGG
 493  D  G  F   Y  E  C   I  F  Q  P   I  F  L   L  S  G   Y  T  M  W   I  R  I   N  H  S   L  G  S  L   D  S  P
                             ^begin13-2
```

08/779457

Figure 1C

08/779457

```
          nlaIII
         nspI
         nspHI            tfiI                         fokI
         aflIII           hinfI    hphI            mnlI   bsrI
1701 ACCAACATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA GTGTGAAAGC AGAAATTACT ATAAACATTG GATTATTGAA AATATCTTGG
     TGGTTGTACA CAGGAAGGAC TAAGACACCA CTTCGGTGAC GGAGGTAGGT CACACTTTCG TCTTTAATGA TATTTGTAAC CTAATAACTT TTATAGAACC
526  P  T  C   V  L  P  D   S  V  V   K  P  L   P  P  S  S   V  K  A   E  I  T   I  N  I  G   L  L  K   I  S  W

                                                  tfiI
                                                  hinfI           tru9I            rsaI
            bsrI                              xcmI                mseI             csp6I    mboII       mnlI       sfaNI
1801 GAAAAGCCAG TCTTTCCAGA GAATAACCTT CAATTCCAGA TTCGCTATGG TTTAAGTGGA AAAGAAGTAC AATGGAAGAT GTATGAGGTT TATGATGCAA
     CTTTTCGGTC AGAAAGGTCT CTTATTGGAA GTTAAGGTCT AAGCGATACC AAATTCACCT TTTCTTCATG TTACCTTCTA CATACTCCAA ATACTACGTT
559  E  K  P  V   E  P  E   N  N  L   Q  F  Q  I   R  Y  G   L  S  G   K  E  V  Q   W  K  M   Y  E  V   Y  D  A  K

                                                                         hinPI           rmaI
                      bsmAI bsrI                                         hhaI/cfoI  mnlI maeI     bsrI
1901 AATCAAAATC TGTCAGTCTC CCAGTTCCAG ACTTGTGTGC AGTCTATGCT GTTCAGGTGC GCTGTAAGAG GCTAGATGGA CTGGGATATT GGAGTAATTG
     TTAGTTTTAG ACAGTCAGAG GGTCAAGGTC TGAACACACG TCAGATACGA CAAGTCCACG CGACATTCTC CGATCTACCT GACCCTATAA CCTCATTAAC
593   S  K  S   V  S  L   P  V  P  D   L  C  A   V  Y  A   V  Q  V  R   C  K  R   L  D  G   L  G  Y  W   S  N  W

                                                               sau96I
                                                               avaII
                                                               asuI
                                                              ppuMI                       tru9I
                                                     ecoNI    eco0109I/draII              mseI
                                  nlaIII             bslI    mnlI     apoI               aseI/asnI/vspI
2001 GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAAGTT CCTATGAGAG GACCTGAATT TTGGAGAATA ATTAATGGAG ATACTATGAA AAAGGAGAAA
     CTCGTTAGGT CGGATGTGTC AACAGTACCT ATATTTTCAA GGATACTCTC CTGGACTTAA AACCTCTTAT TAATTACCTC TATGATACTT TTTCCTCTTT
626   S  N  P   A  Y  T  V   V  M  D   I  K  V   P  M  R  G   P  E  F   W  R  I   I  N  G  D   T  M  K   K  E  K

                                                pleI
        maeIII                                  hinfI                                                            nlaIII
2101 AATGTCACTT TACTTTGGAA GCCCCTGATG AAAAATGACT CATTGTGCAG TGTTCAGAGA TATGTGATAA ACCATCATAC TTCCTGCAAT GGAACATGGT
     TTACAGTGAA ATGAAACCTT CGGGGACTAC TTTTTACTGA GTAACACGTC ACAAGTCTCT ATACACTATT TGGTAGTATG AAGGACGTTA CCTTGTACCA
659  N  V  T  L   L  W  K   P  L  M   K  N  D  S   L  C  S   V  Q  R   Y  V  I  N   H  H  T   S  C  N   G  T  W  S

                                                                                         haeIII/palI
                                                                                       mscI/balI
                                                                                       haeI
                                                                                       eaeI
       mboII                    apoI                                          maeIII   cfrI          munI
2201 CAGAAGATGT GGGAAATCAC ACGAAATTCA CTTTCCTGTG GACAGAGCAA GCACATACTG TTACGGTTCT GGCCATCAAT TCAATTGGTG CTTCTGTTGC
     GTCTTCTACA CCCTTTAGTG TGCTTTAAGT GAAAGGACAC CTGTCTCGTT CGTGTATGAC AATGCCAAGA CCGGTAGTTA AGTTAACCAC GAAGACAACG
693   E  D  V   G  N  H   T  K  F  T   F  L  W   T  E  Q   A  H  T  V   T  V  L   A  I  N   S  I  G  A   S  V  A
```

Figure 1D

08/779457

```
                                                                                           scrFI
                                                                                           mvaI
                                                                                           ecoRII
                                                                                           dsaV
                                                                                           bstNI
                 bslI     haeIII/palI          ddeI         tru9I                          apyI[dcm+]
      tru9I tru9I         haeI                 draIII       mseI
   apoI mseI mseI         nlaIII               maeIII     ahaIII/draI
2301 AAATTTTAAT TTAACCTTTT CATGGCCTAT GAGCAAAGTA AATATCGTGC AGTCACTCAG TGCTTATCCT TTAAACAGCA GTTGTGTGAT TGTTTCCTGG
     TTTAAAATTA AATTGGAAAA GTACCGGATA CTCGTTTCAT TTATAGCACG TCAGTGAGTC ACGAATAGGA AATTTGTCGT CAACACACTA ACAAAGGACC
726   N  F  N   L  T  F  S   W  P  M   S  K  V   N  I  V  Q   S  L  S   A  Y  P   L  N  S  S   C  V  I   V  S  W

                                                                                    xmnI
                                                                                    tfiI
                                                                                    hinfI mboII
                                                                                    asp700
      draIII                                               tru9I                 ddeI   earI/ksp632I
     hphI bsrI            aluI                             mseI  mboII hphI
2401 ATACTATCAC CCAGTGATTA CAAGCTAATG TATTTTATTA TTGAGTGGAA AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG
     TATGATAGTG GGTCACTAAT GTTCGATTAC ATAAAATAAT AACTCACCTT TTTAGAATTA CTTCTACCAC TTTATTTTAC CGAATCTTAG AGAAGTAGAC
759  I  L  S  P   S  D  Y   K  L  M   Y  F  I  I   E  W  K   N  L  N   E  D  G  E   I  K  W   L  R  I   S  S  S  V

                          sau3AI
                          mboI/ndeII[dam-]
                          dpnI[dam+]
                          dpnII[dam-]                bsrI
     tru9I               bclI[dam-]               rsaI                                                         tru9I
     mseI               nlaIII                    csp6I                 sspI                                  mseI
                                                                                                            aseI/asnI/vspI
2501 TTAAGAAGTA TTATATCCAT GATCATTTTA TCCCCATTGA GAAGTACCAG TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAAAC CAAAGATAAT
     AATTCTTCAT AATATAGGTA CTAGTAAAAT AGGGGTAACT CTTCATGGTC AAGTCAGAAA TGGGTTATAA ATACCTTCCT CACCCTTTTG GTTTCTATTA
793    K  K  Y   Y  I  H   D  H  F  I   P  I  E   K  Y  Q   F  S  L  Y   P  I  F   M  E  G   V  G  K  P   K  I  I

                                                                                 mboII
                                                    bspMI                         earI/ksp632I
                                                   sfaNI             bsrI        mnlI
2601 TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT TATATGTAAT TGTGCCAGTA ATTATTTCCT CTTCCATCTT ATTGCTTGGA
     ATTATCAAAG TGAGTTCTAC TATAACTTTT TGTGGTCTCA CTACGTCCAA ATATACATTA ACACGGTCAT TAATAAAGGA GAAGGTAGAA TAACGAACCT
826   N  S  F   T  Q  D  D   I  E  K   H  Q  S   D  A  G  L   Y  V  I   V  P  V   I  I  S  S   S  I  L   L  L  G

                                                                                   bsp1286
                                                                                   bmyI
                                                                                 scrFI
                                                                                 mvaI
                                                                                 ecoRII
                                                                                 dsaV
                                                                                 bstNI
        tru9I                                       xmnI                         bsaJI         tru9I
        mseI                                        mboII                        apyI[dcm+]    mseI
      aseI/asnI/vspI                   aluI         asp700
2701 ACATTATTAA TATCACACCA AAGAATGAAA AAGCTATTTT GGGAAGATGT TCCGAACCCC AAGAATTGTT CCTGGGCACA AGGACTTAAT TTTCAGAAGC
     TGTAATAATT ATAGTGTGGT TTCTTACTTT TTCGATAAAA CCCTTCTACA AGGCTTGGGG TTCTTAACAA GGACCCGTGT TCCTGAATTA AAAGTCTTCG
859  T  L  L  I   S  H  Q   R  M  K   K  L  F  W   E  D  V   P  N  P   K  N  C  S   W  A  Q   G  L  N   F  Q  K  P
```

Figure 1E

08/779457

```
                                       nlaIII
                                      nspI      earI/ksp632I
                                      nspHI  sau96I
                                      aflIII avaII
                                     eam1105I  mnlI                               ecoRV
        maeII                 sfaNI  maeIII   asuI mboII   nlaIV                mboII
       psp1406I  sfaNI
2801 CAGAAACGTT TGAGCATCTT TTTATCAAGC ATACAGCATC AGTGACATGT GGTCCTCTTC TTTTGGAGCC TGAAACAATT TCAGAAGATA TCAGTGTTGA
     GTCTTTGCAA ACTCGTAGAA AAATAGTTCG TATGTCGTAG TCACTGTACA CCAGGAGAAG AAAACCTCGG ACTTTGTTAA AGTCTTCTAT AGTCACAACT
893   E  T  F   E  H  L   F  I  K  H   T  A  S   V  T  C   G  P  L  L   L  E  P   E  T  I   S  E  D  I   S  V  D

                                                            sau3AI
                                                            mboI/ndeII[dam-]
                                                            dpnI[dam+]
                                                            dpnII[dam-]
                                                           bstYI/xhoII                          bsrI
                                      bsmAI                bglII                               maeIII
        nlaIII             sfaNI       bsaI
2901 TACATCATGG AAAAATAAAG ATGAGATGAT GCCAACAACT GTGGTCTCTC TACTTTCAAC AACAGATCTT GAAAAGGGTT CTGTTTGTAT TAGTGACCAG
     ATGTAGTACC TTTTTATTTC TACTCTACTA CGGTTGTTGA CACCAGAGAG ATGAAAGTTG TTGTCTAGAA CTTTTCCCAA GACAAACATA ATCACTGGTC
926  T  S  W   K  N  K  D   E  M  M   P  T  T   V  V  S  L   L  S  T   T  D  L   E  K  G  S   V  C  I   S  D  Q

                                                                                             sau3AI
                                                                                             mboI/ndeII[dam-]
           tru9I                                                                             dpnI[dam+]
           mseI                   ddeI maeIII                                    tru9I       dpnII[dam-]
          hpaI       ddeI   ddeI  rsaI mnlI                                      mseI       bclI[dam-]
          hincII/hindII mnlI mnlI csp6I bstEII    mnlI          bsmAI
3001 TTCAACAGTG TTAACTTCTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTTGTTAA ATACGCCACG CTGATCAGCA
     AAGTTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGGATACT CCTGCTTTCG GTCTCTGTTG GGAAACAATT TATGCGGTGC GACTAGTCGT
959 F  N  S  V   N  F  S   E  A  E   G  T  E  V   T  Y  E   D  E  S   Q  R  Q  P   F  V  K   Y  A  T   L  I  S  N

                                                 draIII
                 hphI                           hphI          rmaI                 tfiI
               bsrI   mboII                    maeIII         maeI     apoI        hinfI
3101 ACTCTAAACC AAGTGAAACT GGTGAAGAAC AAGGGCTTAT AAATAGTTCA GTCACCAAGT GCTTCTCTAG CAAAAATTCT CCGTTGAAGG ATTCTTTCTC
     TGAGATTTGG TTCACTTTGA CCACTTCTTG TTCCCGAATA TTTATCAAGT CAGTGGTTCA CGAAGAGATC GTTTTTAAGA GGCAACTTCC TAAGAAAGAG
993   S  K  P   S  E  T   G  E  E  Q   G  L  I   N  S  S   V  T  K  C   F  S  S   K  N  S   P  L  K  D   S  F  S

                       scrFI
                       mvaI
                       ecoRII
                       dsaV                    sfaNI
                       bstNI                   sau3AI fokI
                       apyI[dcm+]              mboI/ndeII[dam-]
                     sau96I               mamI[dam-]
                     haeIII/palI               dpnI[dam+]
         nlaIII      asuI                      dpnII[dam-]
      aluI         mnlI bsaJI                  bsaBI[dam-]    hphI    mnlI      ddeI        fokI
3201 TAATAGCTCA TGGGAGATAG AGGCCCAGGC ATTTTTTATA TTATCAGATC AGCATCCCAA CATAATTTCA CCACACCTCA CATTCTCAGA AGGATTGGAT
     ATTATCGAGT ACCCTCTATC TCCGGGTCCG TAAAAAATAT AATAGTCTAG TCGTAGGGTT GTATTAAAGT GGTGTGGAGT GTAAGAGTCT TCCTAACCTA
1026 N  S  S   W  E  I  E   A  Q  A   F  F  I   L  S  D  Q   H  P  N   I  I  S   P  H  L  T   F  S  E   G  L  D
```

Figure 1F

08/779457

```
                                                                                     mnlI
                                                                                 hphI
                                                                               maeIII
                                     mboII                                    bstEII
                    mnlI   apoI   eco57I
3301 GAACTTTTGA AATTGGAGGG AAATTTCCCT GAAGAAAATA ATGATAAAAA GTCTATCTAT TATTTAGGGG TCACCTCAAT CAAAAAGAGA GAGAGTGGTG
     CTTGAAAACT TTAACCTCCC TTTAAAGGGA CTTCTTTTAT TACTATTTTT CAGATAGATA ATAAATCCCC AGTGGAGTTA GTTTTTCTCT CTCTCACCAC
1059 E  L  L  K   L  E  G   N  F  P   E  E  N  N   D  K  K   S  I  Y   Y  L  G  V   T  S  I   K  K  R   E  S  G  V

                                                                                      scrFI
                                                                                      mvaI
                                                                                      ecoRII
                                                                                      dsaV
                                                                                      bstNI
                                     bsp1286                                          apyI[dcm+]
          drdI                       bmyI                                           gsuI/bpmI
3401 TGCTTTTGAC TGACAAGTCA AGGGTATCGT GCCCATTCCC AGCCCCCTGT TTATTCACGG ACATCAGAGT TCTCCAGGAC AGTTGCTCAC ACTTTGTAGA
     ACGAAAACTG ACTGTTCAGT TCCCATAGCA CGGGTAAGGG TCGGGGGACA AATAAGTGCC TGTAGTCTCA AGAGGTCCTG TCAACGAGTG TGAAACATCT
1093  L  L  T   D  K  S   R  V  S  C   P  F  P   A  P  C   L  F  T  D   I  R  V   L  Q  D   S  C  S  H   F  V  E

                                                                                                  nlaIII
                                                                                                sau3AI
                                   mboII             nlaIII                          pleI       mboI/ndeII[dam-]
                            rmaI   bpuAI             nspI                            hinfI      dpnI[dam+]
                ddeI        maeI   bbsI       sfaNI  nspHI mnlI                   ddeI          dpnII[dam-]
3501 AAATAATATC AACTTAGGAA CTTCTAGTAA GAAGACTTTT GCATCTTACA TGCCTCAATT CCAAACTTGT TCTACTCAGA CTCATAAGAT CATGGAAAAC
     TTTATTATAG TTGAATCCTT GAAGATCATT CTTCTGAAAA CGTAGAATGT ACGGAGTTAA GGTTTGAACA AGATGAGTCT GAGTATTCTA GTACCTTTTG
1126  N  N  I   N  L  G  T   S  S  K   K  T  F   A  S  Y  M   P  Q  F   Q  T  C   S  T  Q  T   H  K  I   M  E  N

                                   mboII
           maeIII               eco57I      eco57I
3601 AAGATGTGTG ACCTAACTGT GTAATTTCAC TGAAGAAACC TTCAGATTTG TGTTATAATG GGTAATATAA AGTGTAATAG ATTATAGTTG TGGGTGGGAG
     TTCTACACAC TGGATTGACA CATTAAAGTG ACTTCTTTGG AAGTCTAAAC ACAATATTAC CCATTATATT TCACATTATC TAATATCAAC ACCCACCCTC
1159 K  M  C  D   L  T  V

                                                                                                         xmnI
                 pleI                                                      ddeI maeIII      apoI        asp700
                 hinfI apoI
3701 AGAGAAAAGA AACCAGAGTC AAATTTGAAA ATAATTGTTC CAAATGAATG TTGTCTGTTT GTTCTCTCTT AGTAACATAG ACAAAAAATT TGAGAAAGCC
     TCTCTTTTCT TTGGTCTCAG TTTAAACTTT TATTAACAAG GTTTACTTAC AACAGACAAA CAAGAGAGAA TCATTGTATC TGTTTTTTAA ACTCTTTCGG

                                                                   sau96I
                                                                   nlaIV
                                                                   avaII
                                 mboII                  rmaI       asuI                rmaI
                              earI/ksp632I              maeI       ppuMI               maeI
                  accI         sapI              aluI              eco0109I/draII      aluI
3801 TTCATAAGCC TACCAATGTA GACACGCTCT TCTATTTTAT TCCCAAGCTC TAGTGGGAAG GTCCCTTGTT TCCAGCTAGA AATAAGCCCA ACAGACACCA
     AAGTATTCGG ATGGTTACAT CTGTGCGAGA AGATAAAATA AGGGTTCGAG ATCACCCTTC CAGGGAACAA AGGTCGATCT TTATTCGGGT TGTCTGTGGT

                                                                                                     nspI
                                                                                                     nspHI
                                                                       rsaI                       tru9I nlaIII
                                   mnlI               mnlI             csp6I                      mseI aflIII
3901 TCTTTTGTGA GATGTAATTG TTTTTTCAGA GGGCGTGTTG TTTTACCTCA AGTTTTTGTT TTGTACCAAC ACACACACAC ACACACATTC TTAACACATG
     AGAAAACACT CTACATTAAC AAAAAAGTCT CCCGCACAAC AAAATGGAGT TCAAAAACAA AACATGGTTG TGTGTGTGTG TGTGTGTAAG AATTGTGTAC
```

Figure 1G

```
                                                                                                              sfuI
                                                                                                              bstBI
                                                                                                              bsiCI
                                                                                                              asuII
                                                                                                 tru9I       ecoRI
                                                                scfI                             mseI        apoI
4001 TCCTTGTGTG TTTTGAGAGT ATATTATGTA TTTATATTTT GTGCTATCAG ACTGTAGGAT TTGAAGTAGG ACTTTCCTAA ATGTTTAAGA TAAACAGAAT
     AGGAACACAC AAAACTCTCA TATAATACAT AAATATAAAA CACGATAGTC TGACATCCTA AACTTCATCC TGAAAGGATT TACAAATTCT ATTTGTCTTA

     taqI
4101 TC
     AG

length: 4102
```

Figure 1H

Signal Peptide

09/779457

wsxfull.6.4.variant	1	MICQKFCVVLLHWEFIYVITAFNLSYPITPWRFKLSCMPPNSTYDYFLLP
wsxfull.12.1.variant	1	MICQKFCVVLLHWEFIYVITAFNLSYPITPWRFKLSCMPPNSTYDYFLLP
wsxfull.13.2.variant	1	MICQKFCVVLLHWEFIYVITAFNLSYPITPWRFKLSCMPPNSTYDYFLLP
wsxfull.6.4.variant	51	AGLSKNTSNSNGHYETAVEPKFNSSGTHFSNLSKTTFHCCFRSEQDRNCS
wsxfull.12.1.variant	51	AGLSKNTSNSNGHYETAVEPKFNSSGTHFSNLSKTTFHCCFRSEQDRNCS
wsxfull.13.2.variant	51	AGLSKNTSNSNGHYETAVEPKFNSSGTHFSNLSKTTFHCCFRSEQDRNCS
wsxfull.6.4.variant	101	LCADNIEGKTFVSTVNSLVFQQIDANWNIQCWLKGDLKLFICYVESLFKN
wsxfull.12.1.variant	101	LCADNIEGKTFVSTVNSLVFQQIDANWNIQCWLKGDLKLFICYVESLFKN
wsxfull.13.2.variant	101	LCADNIEGKTFVSTVNSLVFQQIDANWNIQCWLKGDLKLFICYVESLFKN
wsxfull.6.4.variant	151	LFRNYNYKVHLLYVLPEVLEDSPLVPQKGSFQMVHCNCSVHECCECLVPV
wsxfull.12.1.variant	151	LFRNYNYKVHLLYVLPEVLEDSPLVPQKGSFQMVHCNCSVHECCECLVPV
wsxfull.13.2.variant	151	LFRNYNYKVHLLYVLPEVLEDSPLVPQKGSFQMVHCNCSVHECCECLVPV
wsxfull.6.4.variant	201	PTAKLNDTLLMCLKITSGGVIFQSPLMSVQPINMVKPDPPLGLHMEITDD
wsxfull.12.1.variant	201	PTAKLNDTLLMCLKITSGGVIFQSPLMSVQPINMVKPDPPLGLHMEITDD
wsxfull.13.2.variant	201	PTAKLNDTLLMCLKITSGGVIFQSPLMSVQPINMVKPDPPLGLHMEITDD
wsxfull.6.4.variant	251	GNLKISWSSPPLVPFPLQYQVKYSENSTTVIREADKIVSATSLLVDSILP
wsxfull.12.1.variant	251	GNLKISWSSPPLVPFPLQYQVKYSENSTTVIREADKIVSATSLLVDSILP
wsxfull.13.2.variant	251	GNLKISWSSPPLVPFPLQYQVKYSENSTTVIREADKIVSATSLLVDSILP
wsxfull.6.4.variant	301	GSSYEVQVRGKRLDGPGIWSDWSTPRVFTTQDVIYFPPKILTSVGSNVSF
wsxfull.12.1.variant	301	GSSYEVQVRGKRLDGPGIWSDWSTPRVFTTQDVIYFPPKILTSVGSNVSF
wsxfull.13.2.variant	301	GSSYEVQVRGKRLDGPGIWSDWSTPRVFTTQDVIYFPPKILTSVGSNVSF
wsxfull.6.4.variant	351	HCIYKKENKIVPSKEIVWWMNLAEKIPQSQYDVVSDHVSKVTFFNLNETK
wsxfull.12.1.variant	351	HCIYKKENKIVPSKEIVWWMNLAEKIPQSQYDVVSDHVSKVTFFNLNETK
wsxfull.13.2.variant	351	HCIYKKENKIVPSKEIVWWMNLAEKIPQSQYDVVSDHVSKVTFFNLNETK
wsxfull.6.4.variant	401	PRGKFTYDAVYCCNEHECHHRYAELYVIDVNINISCETDGYLTKMTCRWS
wsxfull.12.1.variant	401	PRGKFTYDAVYCCNEHECHHRYAELYVIDVNINISCETDGYLTKMTCRWS
wsxfull.13.2.variant	401	PRGKFTYDAVYCCNEHECHHRYAELYVIDVNINISCETDGYLTKMTCRWS
wsxfull.6.4.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHPISEPKDCYLQSDGFYECIF
wsxfull.12.1.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHPISEPKDCYLQSDGFYECIF
wsxfull.13.2.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHPISEPKDCYLQSDGFYECIF
wsxfull.6.4.variant	501	QPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPPSSVKAEITIN
wsxfull.12.1.variant	501	QPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPPSSVKAEITIN
wsxfull.13.2.variant	501	QPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPPSSVKAEITIN
wsxfull.6.4.variant	551	IGLLKISWEKPVFPENNLQFQIRYGLSGKEVQWKMYEVYDAKSKSVSLPV
wsxfull.12.1.variant	551	IGLLKISWEKPVFPENNLQFQIRYGLSGKEVQWKMYEVYDAKSKSVSLPV
wsxfull.13.2.variant	551	IGLLKISWEKPVFPENNLQFQIRYGLSGKEVQWKMYEVYDAKSKSVSLPV
wsxfull.6.4.variant	601	PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
wsxfull.12.1.variant	601	PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
wsxfull.13.2.variant	601	PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
wsxfull.6.4.variant	651	GDTMKKEKNVTLLWKPLMKNDSLCSVQRYVINHHTSCNGTWSEDVGNHTK
wsxfull.12.1.variant	651	GDTMKKEKNVTLLWKPLMKNDSLCSVQRYVINHHTSCNGTWSEDVGNHTK
wsxfull.13.2.variant	651	GDTMKKEKNVTLLWKPLMKNDSLCSVQRYVINHHTSCNGTWSEDVGNHTK
wsxfull.6.4.variant	701	FTFLWTEQAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVQSLSAYPLN
wsxfull.12.1.variant	701	FTFLWTEQAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVQSLSAYPLN
wsxfull.13.2.variant	701	FTFLWTEQAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVQSLSAYPLN

Figure 2A

```
                                                                                       Trans··
wsxfull.6.4.variant   751  SSCVIVSWILSPSDYKLMYFIIEWKNLNEDGEIKWLRISSSVKKYYIHDH
wsxfull.12.1.variant  751  SSCVIVSWILSPSDYKLMYFIIEWKNLNEDGEIKWLRISSSVKKYYIHDH
wsxfull.13.2.variant  751  SSCVIVSWILSPSDYKLMYFIIEWKNLNEDGEIKWLRISSSVKKYYIHDH

wsxfull.6.4.variant   801  FIPIEKYQFSLYPIFMEGVGKPKIINSFTQDDIEKHQSDAGLYVIVPVII
wsxfull.12.1.variant  801  FIPIEKYQFSLYPIFMEGVGKPKIINSFTQDDIEKHQSDAGLYVIVPVII
wsxfull.13.2.variant  801  FIPIEKYQFSLYPIFMEGVGKPKIINSFTQDDIEKHQSDAGLYVIVPVII

                           membrane Domain        Box 1
wsxfull.6.4.variant   851  SSSILLLGTLLISHQRMKKLFWEDVPNPKNCSWAQGLNFQK.........
wsxfull.12.1.variant  851  SSSILLLGTLLISHQRMKKLFWEDVPNPKNCSWAQGLNFQK......MF.
wsxfull.13.2.variant  851  SSSILLLGTLLISHQRMKKLFWEDVPNPKNCSWAQGLNFQKPETFEHLFI

                                                  Box 2
wsxfull.13.2.variant  901  KHTASVTCGPLLLEPETISEDISVDTSWKNKDEMMPTTVVSLLSTTDLEK
                                                Box 3
wsxfull.13.2.variant  951  GSVCISDQFNSVNFSEAEGTEVTYEDESQRQPFVKYATLISNSKPSETGE

wsxfull.6.4.variant   892  .................................................R
wsxfull.12.1.variant  894  .........................................RTPRIVPGH
wsxfull.13.2.variant 1001  EQGLINSSVTKCFSSKNSPLKDSFSNSSWEIEAQAFFILSDQHPNIISPH

wsxfull.6.4.variant   893  .......................................TDIL.......
wsxfull.12.1.variant  903  .......................................KDLIF......
wsxfull.13.2.variant 1051  LTFSEGLDELLKLEGNFPEENNDKKSIYYLGVTSIKKRESGVLLTDKSRV

wsxfull.12.1.variant  908  ............RRCLKAACSLRVITTP......................
wsxfull.13.2.variant 1101  SCPFPAPCLFTDIRVLQDSCSHFVENNINLGTSSKKTFASYMPQFQTCST

wsxfull.13.2.variant 1151  QTHKIMENKMCDLTV
```

Figure 2B

```
wsxfull.6.4.variant    1   GAATTCCGGGTTAAAGCTCTCGTGGCATTATCCTTCAGTGGGGCTATTGG

wsxfull.6.4.variant    51  ACTGACTTTTCTTATGCTGGGATGTGCCTTAGAGGATTATGGATTTGCCA
wsxfull.12.1.variant   1   .....................................GAATTCTCGAGTC
wsxfull.13.2.variant   1   .....................................GAATTCTCGAGTC

wsxfull.6.4.variant    101 GTTCACCCTGACCATCTTGAAAATAAGTTATCTCTGATCTCTGTCTGTAT
wsxfull.12.1.variant   14  GACGGCGGGCGTTAAAGCTCTCGTGGCATTATCCTTCAGTGGGGCTATTG
wsxfull.13.2.variant   14  GACGGCGGGCGTTAAAGCTCTCGTGGCATTATCCTTCAGTGGGGCTATTG

wsxfull.6.4.variant    151 GTTACTTCTCTCCCCTCACCAATGGAGAACAAATGTGGGCAAAGTGTACT
wsxfull.12.1.variant   64  GACTGACTTTTCTTATGCTGGGATGTGCCTTAGAGGATTATGGGTGTACT
wsxfull.13.2.variant   64  GACTGACTTTTCTTATGCTGGGATGTGCCTTAGAGGATTATGGGTGTACT

wsxfull.6.4.variant    201 TCTCTGAAGTAAGATGATTTGTCAAAAATTCTGTGTGGTTTTGTTACATT
wsxfull.12.1.variant   114 TCTCTGAAGTAAGATGATTTGTCAAAAATTCTGTGTGGTTTTGTTACATT
wsxfull.13.2.variant   114 TCTCTGAAGTAAGATGATTTGTCAAAAATTCTGTGTGGTTTTGTTACATT

wsxfull.6.4.variant    251 GGGAATTTATTTATGTGATAACTGCGTTTAACTTGTCATATCCAATTACT
wsxfull.12.1.variant   164 GGGAATTTATTTATGTGATAACTGCGTTTAACTTGTCATATCCAATTACT
wsxfull.13.2.variant   164 GGGAATTTATTTATGTGATAACTGCGTTTAACTTGTCATATCCAATTACT

wsxfull.6.4.variant    301 CCTTGGAGATTTAAGTTGTCTTGCATGCCACCAAATTCAACCTATGACTA
wsxfull.12.1.variant   214 CCTTGGAGATTTAAGTTGTCTTGCATGCCACCAAATTCAACCTATGACTA
wsxfull.13.2.variant   214 CCTTGGAGATTTAAGTTGTCTTGCATGCCACCAAATTCAACCTATGACTA

wsxfull.6.4.variant    351 CTTCCTTTTGCCTGCTGGACTCTCAAAGAATACTTCAAATTCGAATGGAC
wsxfull.12.1.variant   264 CTTCCTTTTGCCTGCTGGACTCTCAAAGAATACTTCAAATTCGAATGGAC
wsxfull.13.2.variant   264 CTTCCTTTTGCCTGCTGGACTCTCAAAGAATACTTCAAATTCGAATGGAC

wsxfull.6.4.variant    401 ATTATGAGACAGCTGTTGAACCTAAGTTTAATTCAAGTGGTACTCACTTT
wsxfull.12.1.variant   314 ATTATGAGACAGCTGTTGAACCTAAGTTTAATTCAAGTGGTACTCACTTT
wsxfull.13.2.variant   314 ATTATGAGACAGCTGTTGAACCTAAGTTTAATTCAAGTGGTACTCACTTT

wsxfull.6.4.variant    451 TCTAACTTATCCAAAACAACTTTCCACTGTTGCTTTCGGAGTGAGCAAGA
wsxfull.12.1.variant   364 TCTAACTTATCCAAAACAACTTTCCACTGTTGCTTTCGGAGTGAGCAAGA
wsxfull.13.2.variant   364 TCTAACTTATCCAAAACAACTTTCCACTGTTGCTTTCGGAGTGAGCAAGA

wsxfull.6.4.variant    501 TAGAAACTGCTCCTTATGTGCAGACAACATTGAAGGAAAGACATTTGTTT
wsxfull.12.1.variant   414 TAGAAACTGCTCCTTATGTGCAGACAACATTGAAGGAAAGACATTTGTTT
wsxfull.13.2.variant   414 TAGAAACTGCTCCTTATGTGCAGACAACATTGAAGGAAAGACATTTGTTT

wsxfull.6.4.variant    551 CHACAGTAAATTCTTTAGTTTTTCAACAAATAGATGCAAACTGGAACATA
wsxfull.12.1.variant   464 CAACAGTAAATTCTTTAGTTTTTCAACAAATAGATGCAAACTGGAACATA
wsxfull.13.2.variant   464 CAACAGTAAATTCTTTAGTTTTTCAACAAATAGATGCAAACTGGAACATA

wsxfull.6.4.variant    601 CAGTGCTGGCTAAAAGGAGACTTAAAATTATTCATCTGTTATGTGGAGTC
wsxfull.12.1.variant   514 CAGTGCTGGCTAAAAGGAGACTTAAAATTATTCATCTGTTATGTGGAGTC
wsxfull.13.2.variant   514 CAGTGCTGGCTAAAAGGAGACTTAAAATTATTCATCTGTTATGTGGAGTC

wsxfull.6.4.variant    651 ATTATTTAAGAATCTATTCAGGAATTATAACTATAAGGTCCATCTTTTAT
wsxfull.12.1.variant   564 ATTATTTAAGAATCTATTCAGGAATTATAACTATAAGGTCCATCTTTTAT
wsxfull.13.2.variant   564 ATTATTTAAGAATCTATTCAGGAATTATAACTATAAGGTCCATCTTTTAT

wsxfull.6.4.variant    701 ATGTTCTGCCTGAAGTGTTAGAAGATTCACCTCTGGTTCCCCAAAAAGGC
wsxfull.12.1.variant   614 ATGTTCTGCCTGAAGTGTTAGAAGATTCACCTCTGGTTCCCCAAAAAGGC
wsxfull.13.2.variant   614 ATGTTCTGCCTGAAGTGTTAGAAGATTCACCTCTGGTTCCCCAAAAAGGC

wsxfull.6.4.variant    751 AGTTTTCAGATGGTTCACTGCAATTGCAGTGTTCATGAATGTTGTGAATG
wsxfull.12.1.variant   664 AGTTTTCAGATGGTTCACTGCAATTGCAGTGTTCATGAATGTTGTGAATG
wsxfull.13.2.variant   664 AGTTTTCAGATGGTTCACTGCAATTGCAGTGTTCATGAATGTTGTGAATG
```

Figure 3A

wsxfull.6.4.variant	801	TCTTGTGCCTGTGCCAACAGCCAAACTCAACGACACTCTCCTTATGTGTT
wsxfull.12.1.variant	714	TCTTGTGCCTGTGCCAACAGCCAAACTCAACGACACTCTCCTTATGTGTT
wsxfull.13.2.variant	714	TCTTGTGCCTGTGCCAACAGCCAAACTCAACGACACTCTCCTTATGTGTT
wsxfull.6.4.variant	851	TGAAAATCACATCTGGTGGAGTAATTTTCCAGTCACCTCTAATGTCAGTT
wsxfull.12.1.variant	764	TGAAAATCACATCTGGTGGAGTAATTTTCCAGTCACCTCTAATGTCAGTT
wsxfull.13.2.variant	764	TGAAAATCACATCTGGTGGAGTAATTTTCCAGTCACCTCTAATGTCAGTT
wsxfull.6.4.variant	901	CAGCCCATAAATATGGTGAAGCCTGATCCACCATTAGGTTTGCATATGGA
wsxfull.12.1.variant	814	CAGCCCATAAATATGGTGAAGCCTGATCCACCATTAGGTTTGCATATGGA
wsxfull.13.2.variant	814	CAGCCCATAAATATGGTGAAGCCTGATCCACCATTAGGTTTGCATATGGA
wsxfull.6.4.variant	951	AATCACAGATGATGGTAATTTAAAGATTTCTTGGTCCAGCCCACCATTGG
wsxfull.12.1.variant	864	AATCACAGATGATGGTAATTTAAAGATTTCTTGGTCCAGCCCACCATTGG
wsxfull.13.2.variant	864	AATCACAGATGATGGTAATTTAAAGATTTCTTGGTCCAGCCCACCATTGG
wsxfull.6.4.variant	1001	TACCATTTCCACTTCAATATCAAGTGAAATATTCAGAGAATTCTACAACA
wsxfull.12.1.variant	914	TACCATTTCCACTTCAATATCAAGTGAAATATTCAGAGAATTCTACAACA
wsxfull.13.2.variant	914	TACCATTTCCACTTCAATATCAAGTGAAATATTCAGAGAATTCTACAACA
wsxfull.6.4.variant	1051	GTTATCAGAGAAGCTGACAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.12.1.variant	964	GTTATCAGAGAAGCTGACAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.13.2.variant	964	GTTATCAGAGAAGCTGACAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.6.4.variant	1101	CAGTATACTTCCTGGGTCTTCGTATGAGGTTCAGGTGAGGGGCAAGAGAC
wsxfull.12.1.variant	1014	CAGTATACTTCCTGGGTCTTCGTATGAGGTTCAGGTGAGGGGCAAGAGAC
wsxfull.13.2.variant	1014	CAGTATACTTCCTGGGTCTTCGTATGAGGTTCAGGTGAGGGGCAAGAGAC
wsxfull.6.4.variant	1151	TGGATGGCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.12.1.variant	1064	TGGATGGCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.13.2.variant	1064	TGGATGGCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.6.4.variant	1201	ACACAAGATGTCATATACTTTCCACCTAAAATTCTGACAAGTGTTGGGTC
wsxfull.12.1.variant	1114	ACACAAGATGTCATATACTTTCCACCTAAAATTCTGACAAGTGTTGGGTC
wsxfull.13.2.variant	1114	ACACAAGATGTCATATACTTTCCACCTAAAATTCTGACAAGTGTTGGGTC
wsxfull.6.4.variant	1251	TAATGTTTCTTTTCACTGCATCTATAAGAAGGAAAACAAGATTGTTCCCT
wsxfull.12.1.variant	1164	TAATGTTTCTTTTCACTGCATCTATAAGAAGGAAAACAAGATTGTTCCCT
wsxfull.13.2.variant	1164	TAATGTTTCTTTTCACTGCATCTATAAGAAGGAAAACAAGATTGTTCCCT
wsxfull.6.4.variant	1301	CAAAAGAGATTGTTTGGTGGATGAATTTAGCTGAGAAAATTCCTCAAAGC
wsxfull.12.1.variant	1214	CAAAAGAGATTGTTTGGTGGATGAATTTAGCTGAGAAAATTCCTCAAAGC
wsxfull.13.2.variant	1214	CAAAAGAGATTGTTTGGTGGATGAATTTAGCTGAGAAAATTCCTCAAAGC
wsxfull.6.4.variant	1351	CAGTATGATGTTGTGAGTGATCATGTTAGCAAAGTTACTTTTTTCAATCT
wsxfull.12.1.variant	1264	CAGTATGATGTTGTGAGTGATCATGTTAGCAAAGTTACTTTTTTCAATCT
wsxfull.13.2.variant	1264	CAGTATGATGTTGTGAGTGATCATGTTAGCAAAGTTACTTTTTTCAATCT
wsxfull.6.4.variant	1401	GAATGAAACCAAACCTCGAGGAAAGTTTACCTATGATGCAGTGTACTGCT
wsxfull.12.1.variant	1314	GAATGAAACCAAACCTCGAGGAAAGTTTACCTATGATGCAGTGTACTGCT
wsxfull.13.2.variant	1314	GAATGAAACCAAACCTCGAGGAAAGTTTACCTATGATGCAGTGTACTGCT
wsxfull.6.4.variant	1451	GCAATGAACATGAATGCCATCATCGCTATGCTGAATTATATGTGATTGAT
wsxfull.12.1.variant	1364	GCAATGAACATGAATGCCATCATCGCTATGCTGAATTATATGTGATTGAT
wsxfull.13.2.variant	1364	GCAATGAACATGAATGCCATCATCGCTATGCTGAATTATATGTGATTGAT
wsxfull.6.4.variant	1501	GTCAATATCAATATCTCATGTGAAACTGATGGGTACTTAACTAAAATGAC
wsxfull.12.1.variant	1414	GTCAATATCAATATCTCATGTGAAACTGATGGGTACTTAACTAAAATGAC
wsxfull.13.2.variant	1414	GTCAATATCAATATCTCATGTGAAACTGATGGGTACTTAACTAAAATGAC

Figure 3B

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wsxfull.6.4.variant   1551 TTGCAGATGGTCAACCAGTACAATCCAGTCACTTGCGGAAAGCACTTTGC
wsxfull.12.1.variant  1464 TTGCAGATGGTCAACCAGTACAATCCAGTCACTTGCGGAAAGCACTTTGC
wsxfull.13.2.variant  1464 TTGCAGATGGTCAACCAGTACAATCCAGTCACTTGCGGAAAGCACTTTGC

wsxfull.6.4.variant   1601 AATTGAGGTATCATAGGAGCAGCCTTTACTGTTCTGATATTCCATCTATT
wsxfull.12.1.variant  1514 AATTGAGGTATCATAGGAGCAGCCTTTACTGTTCTGATATTCCATCTATT
wsxfull.13.2.variant  1514 AATTGAGGTATCATAGGAGCAGCCTTTACTGTTCTGATATTCCATCTATT

wsxfull.6.4.variant   1651 CATCCCATATCTGAGCCCAAAGATTGCTATTTGCAGAGTGATGGTTTTTA
wsxfull.12.1.variant  1564 CATCCCATATCTGAGCCCAAAGATTGCTATTTGCAGAGTGATGGTTTTTA
wsxfull.13.2.variant  1564 CATCCCATATCTGAGCCCAAAGATTGCTATTTGCAGAGTGATGGTTTTTA

wsxfull.6.4.variant   1701 TGAATGCATTTTCCAGCCAATCTTCCTATTATCTGGCTACACAATGTGGA
wsxfull.12.1.variant  1614 TGAATGCATTTTCCAGCCAATCTTCCTATTATCTGGCTACACAATGTGGA
wsxfull.13.2.variant  1614 TGAATGCATTTTCCAGCCAATCTTCCTATTATCTGGCTACACAATGTGGA

wsxfull.6.4.variant   1751 TTAGGATCAATCACTCTCTAGGTTCACTTGACTCTCCACCAACATGTGTC
wsxfull.12.1.variant  1664 TTAGGATCAATCACTCTCTAGGTTCACTTGACTCTCCACCAACATGTGTC
wsxfull.13.2.variant  1664 TTAGGATCAATCACTCTCTAGGTTCACTTGACTCTCCACCAACATGTGTC

wsxfull.6.4.variant   1801 CTTCCTGATTCTGTGGTGAAGCCACTGCCTCCATCCAGTGTGAAAGCAGA
wsxfull.12.1.variant  1714 CTTCCTGATTCTGTGGTGAAGCCACTGCCTCCATCCAGTGTGAAAGCAGA
wsxfull.13.2.variant  1714 CTTCCTGATTCTGTGGTGAAGCCACTGCCTCCATCCAGTGTGAAAGCAGA

wsxfull.6.4.variant   1851 AATTACTATAAACATTGGATTATTGAAAATATCTTGGGAAAAGCCAGTCT
wsxfull.12.1.variant  1764 AATTACTATAAACATTGGATTATTGAAAATATCTTGGGAAAAGCCAGTCT
wsxfull.13.2.variant  1764 AATTACTATAAACATTGGATTATTGAAAATATCTTGGGAAAAGCCAGTCT

wsxfull.6.4.variant   1901 TTCCAGAGAATAACCTTCAATTCCAGATTCGCTATGGTTTAAGTGGAAAA
wsxfull.12.1.variant  1814 TTCCAGAGAATAACCTTCAATTCCAGATTCGCTATGGTTTAAGTGGAAAA
wsxfull.13.2.variant  1814 TTCCAGAGAATAACCTTCAATTCCAGATTCGCTATGGTTTAAGTGGAAAA

wsxfull.6.4.variant   1951 GAAGTACAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAATCTGT
wsxfull.12.1.variant  1864 GAAGTACAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAATCTGT
wsxfull.13.2.variant  1864 GAAGTACAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAATCTGT

wsxfull.6.4.variant   2001 CAGTCTCCCAGTTCCAGACTTGTGTGCAGTCTATGCTGTTCAGGTGCGCT
wsxfull.12.1.variant  1914 CAGTCTCCCAGTTCCAGACTTGTGTGCAGTCTATGCTGTTCAGGTGCGCT
wsxfull.13.2.variant  1914 CAGTCTCCCAGTTCCAGACTTGTGTGCAGTCTATGCTGTTCAGGTGCGCT

wsxfull.6.4.variant   2051 GTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATCCAGCC
wsxfull.12.1.variant  1964 GTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATCCAGCC
wsxfull.13.2.variant  1964 GTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATCCAGCC

wsxfull.6.4.variant   2101 TACACAGTTGTCATGGATATAAAAGTTCCTATGAGAGGACCTGAATTTTG
wsxfull.12.1.variant  2014 TACACAGTTGTCATGGATATAAAAGTTCCTATGAGAGGACCTGAATTTTG
wsxfull.13.2.variant  2014 TACACAGTTGTCATGGATATAAAAGTTCCTATGAGAGGACCTGAATTTTG

wsxfull.6.4.variant   2151 GAGAATAATTAATGGAGATACTATGAAAAAGGAGAAAAATGTCACTTTAC
wsxfull.12.1.variant  2064 GAGAATAATTAATGGAGATACTATGAAAAAGGAGAAAAATGTCACTTTAC
wsxfull.13.2.variant  2064 GAGAATAATTAATGGAGATACTATGAAAAAGGAGAAAAATGTCACTTTAC

wsxfull.6.4.variant   2201 TTTGGAAGCCCCTGATGAAAAATGACTCATTGTGCAGTGTTCAGAGATAT
wsxfull.12.1.variant  2114 TTTGGAAGCCCCTGATGAAAAATGACTCATTGTGCAGTGTTCAGAGATAT
wsxfull.13.2.variant  2114 TTTGGAAGCCCCTGATGAAAAATGACTCATTGTGCAGTGTTCAGAGATAT

wsxfull.6.4.variant   2251 GTGATAAACCATCATACTTCCTGCAATGGAACATGGTCAGAAGATGTGGG
wsxfull.12.1.variant  2164 GTGATAAACCATCATACTTCCTGCAATGGAACATGGTCAGAAGATGTGGG
wsxfull.13.2.variant  2164 GTGATAAACCATCATACTTCCTGCAATGGAACATGGTCAGAAGATGTGGG
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Figure 3C

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wsxfull.6.4.variant   2301 AAATCACACGAAATTCACTTTCCTGTGGACAGAGCAAGCACATACTGTTA
wsxfull.12.1.variant  2214 AAATCACACGAAATTCACTTTCCTGTGGACAGAGCAAGCACATACTGTTA
wsxfull.13.2.variant  2214 AAATCACACGAAATTCACTTTCCTGTGGACAGAGCAAGCACATACTGTTA

wsxfull.6.4.variant   2351 CGGTTCTGGCCATCAATTCAATTGGTGCTTCTGTTGCAAATTTTAATTTA
wsxfull.12.1.variant  2264 CGGTTCTGGCCATCAATTCAATTGGTGCTTCTGTTGCAAATTTTAATTTA
wsxfull.13.2.variant  2264 CGGTTCTGGCCATCAATTCAATTGGTGCTTCTGTTGCAAATTTTAATTTA

wsxfull.6.4.variant   2401 ACCTTTTCATGGCCTATGAGCAAAGTAAATATCGTGCAGTCACTCAGTGC
wsxfull.12.1.variant  2314 ACCTTTTCATGGCCTATGAGCAAAGTAAATATCGTGCAGTCACTCAGTGC
wsxfull.13.2.variant  2314 ACCTTTTCATGGCCTATGAGCAAAGTAAATATCGTGCAGTCACTCAGTGC

wsxfull.6.4.variant   2451 TTATCCTTTAAACAGCAGTTGTGTGATTGTTTCCTGGATACTATCACCCA
wsxfull.12.1.variant  2364 TTATCCTTTAAACAGCAGTTGTGTGATTGTTTCCTGGATACTATCACCCA
wsxfull.13.2.variant  2364 TTATCCTTTAAACAGCAGTTGTGTGATTGTTTCCTGGATACTATCACCCA

wsxfull.6.4.variant   2501 GTGATTACAAGCTAATGTATTTTATTATTGAGTGGAAAAATCTTAATGAA
wsxfull.12.1.variant  2414 GTGATTACAAGCTAATGTATTTTATTATTGAGTGGAAAAATCTTAATGAA
wsxfull.13.2.variant  2414 GTGATTACAAGCTAATGTATTTTATTATTGAGTGGAAAAATCTTAATGAA

wsxfull.6.4.variant   2551 GATGGTGAAATAAAATGGCTTAGAATCTCTTCATCTGTTAAGAAGTATTA
wsxfull.12.1.variant  2464 GATGGTGAAATAAAATGGCTTAGAATCTCTTCATCTGTTAAGAAGTATTA
wsxfull.13.2.variant  2464 GATGGTGAAATAAAATGGCTTAGAATCTCTTCATCTGTTAAGAAGTATTA

wsxfull.6.4.variant   2601 TATCCATGATCATTTTATCCCCATTGAGAAGTACCAGTTCAGTCTTTACC
wsxfull.12.1.variant  2514 TATCCATGATCATTTTATCCCCATTGAGAAGTACCAGTTCAGTCTTTACC
wsxfull.13.2.variant  2514 TATCCATGATCATTTTATCCCCATTGAGAAGTACCAGTTCAGTCTTTACC

wsxfull.6.4.variant   2651 CAATATTTATGGAAGGAGTGGGAAAACCAAAGATAATTAATAGTTTCACT
wsxfull.12.1.variant  2564 CAATATTTATGGAAGGAGTGGGAAAACCAAAGATAATTAATAGTTTCACT
wsxfull.13.2.variant  2564 CAATATTTATGGAAGGAGTGGGAAAACCAAAGATAATTAATAGTTTCACT

wsxfull.6.4.variant   2701 CAAGATGATATTGAAAAACACCAGAGTGATGCAGGTTTATATGTAATTGT
wsxfull.12.1.variant  2614 CAAGATGATATTGAAAAACACCAGAGTGATGCAGGTTTATATGTAATTGT
wsxfull.13.2.variant  2614 CAAGATGATATTGAAAAACACCAGAGTGATGCAGGTTTATATGTAATTGT

wsxfull.6.4.variant   2751 GCCAGTAATTATTTCCTCTTCCATCTTATTGCTTGGAACATTATTAATAT
wsxfull.12.1.variant  2664 GCCAGTAATTATTTCCTCTTCCATCTTATTGCTTGGAACATTATTAATAT
wsxfull.13.2.variant  2664 GCCAGTAATTATTTCCTCTTCCATCTTATTGCTTGGAACATTATTAATAT

wsxfull.6.4.variant   2801 CACACCAAAGAATGAAAAAGCTATTTTGGGAAGATGTTCCGAACCCCAAG
wsxfull.12.1.variant  2714 CACACCAAAGAATGAAAAAGCTATTTTGGGAAGATGTTCCGAACCCCAAG
wsxfull.13.2.variant  2714 CACACCAAAGAATGAAAAAGCTATTTTGGGAAGATGTTCCGAACCCCAAG

wsxfull.6.4.variant   2851 AATTGTTCCTGGGCACAAGGACTTAATTTTCAGAAGAGAACGGACATTCT
wsxfull.12.1.variant  2764 AATTGTTCCTGGGCACAAGGACTTAATTTTCAGAAGATGTTCCGAACCCC
wsxfull.13.2.variant  2764 AATTGTTCCTGGGCACAAGGACTTAATTTTCAGAAGCCAGAAACGTTTGA

wsxfull.6.4.variant   2901 TTGAAGTCTAATCATGATCACTACAGATGAACCCAATGTGCCAACTTCCC
wsxfull.12.1.variant  2814 AAGAATTGTTCCTGGGCACAAGGACTTAATTTTCAGAAGATGCTTGAAGG
wsxfull.13.2.variant  2814 GCATCTTTTTATCAAGCATACAGCATCAGTGACATGTGGTCCTCTTCTTT

wsxfull.6.4.variant   2951 AACAGTCTATAGAGTATTAGAAGATTTTTACATTTTGAAGAAGGGCCGGA
wsxfull.12.1.variant  2864 CAGCATGTTCGTTAAGAGTCATCACCACTCCCTAATCTCAAGTACCCAGG
wsxfull.13.2.variant  2864 TGGAGCCTGAAACAATTTCAGAAGATATCAGTGTTGATACATCATGGAAA

wsxfull.6.4.variant   3001 ATTC..............................................
wsxfull.12.1.variant  2914 GACACAAACACTGCGGAAGGCCACAGGGTCCTCTGCATAGGAAAACCAGA
wsxfull.13.2.variant  2914 AATAAAGATGAGATGATGCCAACAACTGTGGTCTCTCTACTTTCAACAAC
```

Figure 3D

08/779457

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wsxfull.12.1.variant 2964 GACCTTTGTTCACTTGTTTATCTGCTGACCCTCCCTCCACTATTGTCCTA
wsxfull.13.2.variant 2964 AGATCTTGAAAAGGGTTCTGTTTGTATTAGTGACCAGTTCAACAGTGTTA

wsxfull.12.1.variant 3014 TGACCCTGCCAAATCCCCCTCTGTGAGAAACACCCAAGAATGATCAATAA
wsxfull.13.2.variant 3014 ACTTCTCTGAGGCTGAGGGTACTGAGGTAACCTATGAGGACGAAAGCCAG

wsxfull.12.1.variant 3064 AAAAAAAAAAAAAAAAAAAAAAAGTCGACTCGAGAATTC...........
wsxfull.13.2.variant 3064 AGACAACCCTTTGTTAAATACGCCACGCTGATCAGCAACTCTAAACCAAG

wsxfull.13.2.variant 3114 TGAAACTGGTGAAGAACAAGGGCTTATAAATAGTTCAGTCACCAAGTGCT

wsxfull.13.2.variant 3164 TCTCTAGCAAAAATTCTCCGTTGAAGGATTCTTTCTCTAATAGCTCATGG

wsxfull.13.2.variant 3214 GAGATAGAGGCCCAGGCATTTTTTATATTATCAGATCAGCATCCCAACAT

wsxfull.13.2.variant 3264 AATTTCACCACACCTCACATTCTCAGAAGGATTGGATGAACTTTTGAAAT

wsxfull.13.2.variant 3314 TGGAGGGAAATTTCCCTGAAGAAAATAATGATAAAAAGTCTATCTATTAT

wsxfull.13.2.variant 3364 TTAGGGGTCACCTCAATCAAAAAGAGAGAGAGTGGTGTGCTTTTGACTGA

wsxfull.13.2.variant 3414 CAAGTCAAGGGTATCGTGCCCATTCCCAGCCCCCTGTTTATTCACGGACA

wsxfull.13.2.variant 3464 TCAGAGTTCTCCAGGACAGTTGCTCACACTTTGTAGAAAATAATATCAAC

wsxfull.13.2.variant 3514 TTAGGAACTTCTAGTAAGAAGACTTTTGCATCTTACATGCCTCAATTCCA

wsxfull.13.2.variant 3564 AACTTGTTCTACTCAGACTCATAAGATCATGGAAAACAAGATGTGTGACC

wsxfull.13.2.variant 3614 TAACTGTGTAATTTCACTGAAGAAACCTTCAGATTTGTGTTATAATGGGT

wsxfull.13.2.variant 3664 AATATAAAGTGTAATAGATTATAGTTGTGGGTGGGAGAGAGAAAAGAAAC

wsxfull.13.2.variant 3714 CAGAGTCAAATTTGAAAATAATTGTTCCAAATGAATGTTGTCTGTTTGTT

wsxfull.13.2.variant 3764 CTCTCTTAGTAACATAGACAAAAAATTTGAGAAAGCCTTCATAAGCCTAC

wsxfull.13.2.variant 3814 CAATGTAGACACGCTCTTCTATTTTATTCCCAAGCTCTAGTGGGAAGGTC

wsxfull.13.2.variant 3864 CCTTGTTTCCAGCTAGAAATAAGCCCAACAGACACCATCTTTTGTGAGAT

wsxfull.13.2.variant 3914 GTAATTGTTTTTTCAGAGGGCGTGTTGTTTTACCTCAAGTTTTTGTTTTG

wsxfull.13.2.variant 3964 TACCAACACACACACACACACACATTCTTAACACATGTCCTTGTGTGTTT

wsxfull.13.2.variant 4014 TGAGAGTATATTATGTATTTATATTTTGTGCTATCAGACTGTAGGATTTG

wsxfull.13.2.variant 4064 AAGTAGGACTTTCCTAAATGTTTAAGATAAACAGAATTC
```

Figure 3E

```
wsxfull.13.2.variant  1   M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
mu.wsx.ecd            1   M M C Q K F Y V V L L H W E F L Y V I A A L N L A Y P I S P W K F K L F C G P P N T T D D S F L S P

wsxfull.13.2.variant  51  A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E Q D R N C S
mu.wsx.ecd            51  A G A P N N A S A L K G A S E A I V E A K F N S S G I Y V P E L S K T V F H C C F G N E Q G Q N C S

wsxfull.13.2.variant  101 L C A D N I E G K T F V S T V N S L V F Q Q I D A N W N I Q C W L K G D L K L F I C Y V E S L F K N
mu.wsx.ecd            101 A L T D N T E G K T L A S V V K A S V F R Q L G V N W D I E C W M K G D L T L F I C H M E P L P K N

wsxfull.13.2.variant  151 L F R N Y N Y K V H L L Y V L P E V L E D S P L V P Q K G S F Q M V H C N C S V H E C C E C L V P V
mu.wsx.ecd            151 P F K N Y D S K V H L L Y Q L P E V I D D S P L P P L K D S F Q T V Q C N C S L R G - C E C H V P V

wsxfull.13.2.variant  201 P T A K L N D T L L M C L K I T S G G V I F Q S P L M S V Q P I N M V K P D P P L G L H M E I T D D
mu.wsx.ecd            200 P R A K L N Y A L L M Y L E I T S A G V S F Q S P L M S L Q P M L V V K P D P P L G L H M E V T D D

wsxfull.13.2.variant  251 G N L K I S W S S P P L V P F P L Q Y Q V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P
mu.wsx.ecd            250 G N L K I S W D S Q T M A P F P L Q Y Q V K Y L E N S - T I V R E A A E I V S A T S L L V D S V L P

wsxfull.13.2.variant  301 G S S Y E V Q V R G K R L D G P G I W S D W S T P R V F T T Q D V I Y F P P K I L T S V G S N V S F
mu.wsx.ecd            299 G S S Y E V Q V R S K R L D G S G V W S D W S S P Q V F T T Q D V V Y F P P K I L T S V G S N A S F

wsxfull.13.2.variant  351 H C I Y K K E N K I V P S K E I V W W M N L A E K I P Q S Q Y D V V S D H V S K V T F F N L N E T K
mu.wsx.ecd            349 H C I Y K N E N Q I V S S K Q I V W W R N L A E K I P E I Q Y S I V S D R V S K V T F S N L K A T R

wsxfull.13.2.variant  401 P R G K F T Y D A V Y C C N E H E C H H R Y A E L Y V I D V N I N I S C E T D G Y L T K M T C R W S
mu.wsx.ecd            399 P R G K F T Y D A V Y C C N E Q A C H H R Y A E L Y V I D V N I N I S C E T D G Y L T K M T C R W S

wsxfull.13.2.variant  451 T S T I Q S L A E S T L Q L R Y H R S S L Y C S D I P S I H P I S E P K D C Y L Q S D G F Y E C I F
mu.wsx.ecd            449 P S T I Q S L V G S T V Q L R Y H R C S L Y C P D S P S I H P T S E P K T A S Y R E T A F M N V F S

wsxfull.13.2.variant  501 Q P I F L L S G Y T M W I R I N H S L G S L D S P P T C V L P D S V V K P L P P S S V K A E I T I N
mu.wsx.ecd            499 S Q S F Y Y L A I Q C G F R I N H S L G S L D S P P T C V L P D S V V K P L P P S N V K A E I T V N

wsxfull.13.2.variant  551 I G L L K I S W E K P V F P E N N L Q F Q I R Y G L S G K E V Q W K M Y E V Y D A K S K S V S L P V
mu.wsx.ecd            549 T G L L K V S W E K P V F P E N N L Q F Q I R Y G L S G K E I Q W K T H E V F D A K S K S A S L L V

wsxfull.13.2.variant  601 P D L C A V Y A V Q V R C K R L D G L G Y W S N W S N P A Y T V V M D I K V P M R G P E F W R I I N
mu.wsx.ecd            599 S D L C A V Y V V Q V R C R R L D G L G Y W S N W S S P A Y T L V M D V K V P M R G P E F W R K M D

wsxfull.13.2.variant  651 G D T M K K E K N V T L L W K P L M K N D S L C S V Q R Y V I N H H T S C N G T W S E D V G N H T K
mu.wsx.ecd            649 G D V T K K E R N V T L L W K P L T K N D S L C S V R R Y V V K H R T A H N G T W S E D V G N R T N

wsxfull.13.2.variant  701 F T F L W T E Q A H T V T V L A I N S I G A S V A N F N L T F S W P M S K V N I V Q S L S A Y P L N
mu.wsx.ecd            699 L T F L W T E P A H T V T V L A V N S L G A S L V N F N L T F S W P M S K V S A V E S L S A Y P L S
```

Figure 4A

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wsxfull.13.2.variant  751  S S C V I V S W I L S P S D Y K L M Y F I I E W K N L N E D G E I K W L R I S [illegible]
mu.wsx.ecd            749  S S C V I L S W T L S P D D Y S L L Y L V I E W K I L N E D D G M K W

wsxfull.13.2.variant  801  F I P I E K Y Q F S L Y P I F M E G V G K P K I I N S F T Q D D I E K H Q S D A G L Y V I V P V I I

wsxfull.13.2.variant  851  S S S I L L L G T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F Q K P E T F E H L F I

wsxfull.13.2.variant  901  K H T A S V T C G P L L L E P E T I S E D I S V D T S W K N K D E M M P T T V V S L L S T T D L E K

wsxfull.13.2.variant  951  G S V C I S D Q F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N S K P S E T G E

wsxfull.13.2.variant  1001 E Q G L I N S S V T K C F S S K N S P L K D S F S N S S W E I E A Q A F F I L S D Q H P N I I S P H

wsxfull.13.2.variant  1051 L T F S E G L D E L L K L E G N F P E E N N D K K S I Y Y L G V T S I K K R E S G V L L T D K S R V

wsxfull.13.2.variant  1101 S C P F P A P C L F T D I R V L Q D S C S H F V E N N I N L G T S S K K T F A S Y M P Q F Q T C S T

wsxfull.13.2.variant  1151 Q T H K I M E N K M C D L T V
```

Figure 4B

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mu.wsx.ecd               1 GGGCCCCCCCTCGAAGTCGACGGTATCGATAAGCTTGATATCGAATTCCG

mu.wsx.ecd              51 GCCGGGACACAGGTGGGACACTCTTTTAGTCCTCAATCCCTGGCGCGAGG

mu.wsx.ecd             101 CCACCCAAGGCAACGCAGGACGCAGGGCGTTTGGGGACCAGGCAGCAGAC

mu.wsx.ecd             151 TGGGGCGGTACCTGCGGAGAGCCACGCAACTTCTCCAGGCCTCTGACTAC

mu.wsx.ecd             201 TTTGGAAACTGCCCGGGGCTGCGACATCAACCCCTTAAGTCCCGGAGGCG

mu.wsx.ecd             251 GAAAGAGGGTGGGTTGGTTTGAAAGACACAAGGAAGAAAAATGTGCTGTG

mu.wsx.ecd             301 GGGCGGGTTAAGTTTCCCACCCTCTTCCCCCTTCCCGAGCAAATTAGAAA

mu.wsx.ecd             351 CAAAACAAATAGAAAAGCCAGCCCTCCGGCCAACCAAAGCCCCAAGCGGA
wsxfull.13.2.variant     1 ..................................GAATTCTCGAGTCGAC

mu.wsx.ecd             401 GCCCCAAGCGGAGCCCCAGCCGGAGCACTCCTTTAAAAGGATTTGCAGCG
wsxfull.13.2.variant    17 GGCGGGCGTTAAAGCTCTCGTGGCATTATCCTTCAGTGGGGCT...ATTG

mu.wsx.ecd             451 GTGAGGAAAAAACCAGACCCGACCGAGGAATCGTTCTGCAAATCCAGGTG
wsxfull.13.2.variant    64 GACTGACTTTTCTTATGCTGGGATGTG....CCTTAGAGGATTATGGGTG

mu.wsx.ecd             501 TACACCTCTGAAGAAAGATGATGTGTCAGAAATTCTATGTGGTTTTGTTA
wsxfull.13.2.variant   110 TACTTCTCTGAAGTAAGATGATTTGTCAAAAATTCTGTGTGGTTTTGTTA

mu.wsx.ecd             551 CACTGGGAATTTCTTTATGTGATAGCTGCACTTAACCTGGCATATCCAAT
wsxfull.13.2.variant   160 CATTGGGAATTTATTTATGTGATAACTGCGTTTAACTTGTCATATCCAAT

mu.wsx.ecd             601 CTCTCCCTGGAAATTTAAGTTGTTTTGTGGACCACCGAACACAACCGATG
wsxfull.13.2.variant   210 TACTCCTTGGAGATTTAAGTTGTCTTGCATGCCACCAAATTCAACCTATG

mu.wsx.ecd             651 ACTCCTTTCTCTCACCTGCTGGAGCCCCAAACAATGCCTCGGCTTTGAAG
wsxfull.13.2.variant   260 ACTACTTCCTTTTGCCTGCTGGACTCTCAAAGAATACTTCAAATTCGAAT

mu.wsx.ecd             701 GGGGCTTCTGAAGCAATTGTTGAAGCTAAATTTAATTCAAGTGGTATCTA
wsxfull.13.2.variant   310 GGACATTATGAGACAGCTGTTGAACCTAAGTTTAATTCAAGTGGTACTCA

mu.wsx.ecd             751 CGTTCCTGAGTTATCCAAAACAGTCTTCCACTGTTGCTTTGGGAATGAGC
wsxfull.13.2.variant   360 CTTTTCTAACTTATCCAAAACAACTTTCCACTGTTGCTTTCGGAGTGAGC
```

Figure 5A

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mu.wsx.ecd              801  AAGGTCAAAACTGCTCTGCACTCACAGACAACACTGAAGGGAAGACACTG
wsxfull.13.2.variant    410  AAGATAGAAACTGCTCCTTATGTGCAGACAACATTGAAGGAAAGACATTT

mu.wsx.ecd              851  GCTTCAGTAGTGAAGGCTTCAGTTTTTCGCCAGCTAGGTGTAAACTGGGA
wsxfull.13.2.variant    460  GTTTCAACAGTAAATTCTTTAGTTTTTCAACAAATAGATGCAAACTGGAA

mu.wsx.ecd              901  CATAGAGTGCTGGATGAAAGGGGACTTGACATTATTCATCTGTCATATGG
wsxfull.13.2.variant    510  CATACAGTGCTGGCTAAAAGGAGACTTAAAATTATTCATCTGTTATGTGG

mu.wsx.ecd              951  AGCCATTACCTAAGAACCCCTTCAAGAATTATGACTCTAAGGTCCATCTT
wsxfull.13.2.variant    560  AGTCATTATTTAAGAATCTATTCAGGAATTATAACTATAAGGTCCATCTT

mu.wsx.ecd              1001 TTATATGATCTGCCTGAAGTCATAGATGATTCGCCTCTGCCCCCACTGAA
wsxfull.13.2.variant    610  TTATATGTTCTGCCTGAAGTGTTAGAAGATTCACCTCTGGTTCCCCAAAA

mu.wsx.ecd              1051 AGACAGCTTTCAGACTGTCCAATGCAACTGCAGTCTTCGGG---GATGTG
wsxfull.13.2.variant    660  AGGCAGTTTTCAGATGGTTCACTGCAATTGCAGTGTTCATGAATGTTGTG

mu.wsx.ecd              1098 AATGTCATGTGCCAGTACCCAGAGCCAAACTCAACTACGCTCTTCTGATG
wsxfull.13.2.variant    710  AATGTCTTGTGCCTGTGCCAACAGCCAAACTCAACGACACTCTCCTTATG

mu.wsx.ecd              1148 TATTTGGAAATCACATCTGCCGGTGTGAGTTTTCAGTCACCTCTGATGTC
wsxfull.13.2.variant    760  TGTTTGAAAATCACATCTGGTGGAGTAATTTTCCAGTCACCTCTAATGTC

mu.wsx.ecd              1198 ACTGCAGCCCATGCTTGTTGTGAAACCCGATCCACCCTTAGGTTTGCATA
wsxfull.13.2.variant    810  AGTTCAGCCCATAAATATGGTGAAGCCTGATCCACCATTAGGTTTGCATA

mu.wsx.ecd              1248 TGGAAGTCACAGATGATGGTAATTTAAAGATTTCTTGGGACAGCCAAACA
wsxfull.13.2.variant    860  TGGAAATCACAGATGATGGTAATTTAAAGATTTCTTGGTCCAGCCCACCA

mu.wsx.ecd              1298 ATGGCACCATTTCCGCTTCAATATCAGGTGAAATATTTAGAGAATTCTAC
wsxfull.13.2.variant    910  TTGGTACCATTTCCACTTCAATATCAAGTGAAATATTCAGAGAATTCTAC

mu.wsx.ecd              1348 AA---TTGTAAGAGAGGCTGCTGAAATTGTCTCAGCTACATCTCTGCTGG
wsxfull.13.2.variant    960  AACAGTTATCAGAGAAGCTGACAAGATTGTCTCAGCTACATCCCTGCTAG

mu.wsx.ecd              1395 TAGACAGTGTGCTTCCTGGATCTTCATATGAGGTCCAGGTGAGGAGCAAG
wsxfull.13.2.variant    1010 TAGACAGTATACTTCCTGGGTCTTCGTATGAGGTTCAGGTGAGGGGCAAG

mu.wsx.ecd              1445 AGACTGGATGGTTCAGGAGTCTGGAGTGACTGGAGTTCACCTCAAGTCTT
wsxfull.13.2.variant    1060 AGACTGGATGGCCCAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTT

mu.wsx.ecd              1495 TACCACACAAGATGTTGTGTATTTTCCACCCAAAATTCTGACTAGTGTTG
wsxfull.13.2.variant    1110 TACCACACAAGATGTCATATACTTTCCACCTAAAATTCTGACAAGTGTTG
```

Figure 5B

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mu.wsx.ecd              1545 GATCGAATGCTTCCTTTCATTGCATCTACAAAAACGAAAACCAGATTGTC
wsxfull.13.2.variant    1160 GGTCTAATGTTTCTTTTCACTGCATCTATAAGAAGGAAAACAAGATTGTT

mu.wsx.ecd              1595 TCCTCAAAACAGATAGTTTGGTGGAGGAATCTAGCTGAGAAAATCCCTGA
wsxfull.13.2.variant    1210 CCCTCAAAAGAGATTGTTTGGTGGATGAATTTAGCTGAGAAAATTCCTCA

mu.wsx.ecd              1645 GATACAGTACAGCATTGTGAGTGACCGAGTTAGCAAAGTTACCTTCTCCA
wsxfull.13.2.variant    1260 AAGCCAGTATGATGTTGTGAGTGATCATGTTAGCAAAGTTACTTTTTTCA

mu.wsx.ecd              1695 ACCTGAAAGCCACCAGACCTCGAGGGAAGTTTACCTATGACGCAGTGTAC
wsxfull.13.2.variant    1310 ATCTGAATGAAACCAAACCTCGAGGAAAGTTTACCTATGATGCAGTGTAC

mu.wsx.ecd              1745 TGCTGCAATGAGCAGGCGTGCCATCACCGCTATGCTGAATTATACGTGAT
wsxfull.13.2.variant    1360 TGCTGCAATGAACATGAATGCCATCATCGCTATGCTGAATTATATGTGAT

mu.wsx.ecd              1795 CGATGTCAATATCAATATATCATGTGAAACTGACGGGTACTTAACTAAAA
wsxfull.13.2.variant    1410 TGATGTCAATATCAATATCTCATGTGAAACTGATGGGTACTTAACTAAAA

mu.wsx.ecd              1845 TGACTTGCAGATGGTCACCCAGCACAATCCAATCACTAGTGGGAAGCACT
wsxfull.13.2.variant    1460 TGACTTGCAGATGGTCAACCAGTACAATCCAGTCACTTGCGGAAAGCACT

mu.wsx.ecd              1895 GTGCAGCTGAGGTATCACAGGTGCAGCCTGTATTGTCCTGATAGTCCATC
wsxfull.13.2.variant    1510 TTGCAATTGAGGTATCATAGGAGCAGCCTTTACTGTTCTGATATTCCATC

mu.wsx.ecd              1945 TATTCATCCTACGTCTGAGCCCAAA-ACTGCGTCTTACAGAGAGACGGCT
wsxfull.13.2.variant    1560 TATTCATCCCATATCTGAGCCCAAAGATTGCTATTTGCAGAGTGATGGTT

mu.wsx.ecd              1994 TTTATGAATGTGTTTTCCAGCCAATCTTTCTATTATCTGGCTATACAATG
wsxfull.13.2.variant    1610 TTTATGAATGCATTTTCCAGCCAATCTTCCTATTATCTGGCTACACAATG

mu.wsx.ecd              2044 TGGATTCAGGATCAACCATTCTTTAGGTTCACTTGACTCGCCACCAACGT
wsxfull.13.2.variant    1660 TGGATT-AGGATCAATCACTCTCTAGGTTCACTTGACTCTCCACCAACAT

mu.wsx.ecd              2094 GTGTCCTTCCTGACTCCGTAGTAAAACCACTACCTCCATCTAACGTAAAA
wsxfull.13.2.variant    1709 GTGTCCTTCCTGATTCTGTGGTGAAGCCACTGCCTCCATCCAGTGTGAAA

mu.wsx.ecd              2144 GCAGAGATTACTGTAAACACTGGATTATTGAAAGTATCTTGGGAAAAGCC
wsxfull.13.2.variant    1759 GCAGAAATTACTATAAACATTGGATTATTGAAAATATCTTGGGAAAAGCC

mu.wsx.ecd              2194 AGTCTTTCCGGAGAATAACCTTCAATTCCAGATTCGATATGGCTTAAGTG
wsxfull.13.2.variant    1809 AGTCTTTCCAGAGAATAACCTTCAATTCCAGATTCGCTATGGTTTAAGTG

mu.wsx.ecd              2244 GAAAAGAAATACAATGGAAGACACATGAGGTATTCGATGCAAAGTCAAAG
wsxfull.13.2.variant    1859 GAAAAGAAGTACAATGGAAGATGTATGAGGTTTATGATGCAAAATCAAAA
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Figure 5C

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mu.wsx.ecd               2294 TCTGCCAGCCTGCTGGTGTCAGACCTCTGTGCAGTCTATGTGGTCCAGGT
wsxfull.13.2.variant     1909 TCTGTCAGTCTCCCAGTTCCAGACTTGTGTGCAGTCTATGCTGTTCAGGT

mu.wsx.ecd               2344 TCGCTGCCGGCGGTTGGATGGACTAGGATATTGGAGTAATTGGAGCAGTC
wsxfull.13.2.variant     1959 GCGCTGTAAGAGGCTAGATGGACTGGGATATTGGAGTAATTGGAGCAATC

mu.wsx.ecd               2394 CAGCCTATACGCTTGTCATGGATGTAAAAGTTCCTATGAGAGGGCCTGAA
wsxfull.13.2.variant     2009 CAGCCTACACAGTTGTCATGGATATAAAAGTTCCTATGAGAGGACCTGAA

mu.wsx.ecd               2444 TTTTGGAGAAAAATGGATGGGGACGTTACTAAAAAGGAGAGAAATGTCAC
wsxfull.13.2.variant     2059 TTTTGGAGAATAATTAATGGAGATACTATGAAAAAGGAGAAAAATGTCAC

mu.wsx.ecd               2494 CTTGCTTTGGAAGCCCCTGACGAAAAATGACTCACTGTGTAGTGTGAGGA
wsxfull.13.2.variant     2109 TTTACTTTGGAAGCCCCTGATGAAAAATGACTCATTGTGCAGTGTTCAGA

mu.wsx.ecd               2544 GGTACGTGGTGAAGCATCGTACTGCCCACAATGGGACGTGGTCAGAAGAT
wsxfull.13.2.variant     2159 GATATGTGATAAACCATCATACTTCCTGCAATGGAACATGGTCAGAAGAT

mu.wsx.ecd               2594 GTGGGAAATCGGACCAATCTCACTTTCCTGTGGACAGAACCAGCGCACAC
wsxfull.13.2.variant     2209 GTGGGAAATCACACGAAATTCACTTTCCTGTGGACAGAGCAAGCACATAC

mu.wsx.ecd               2644 TGTTACAGTTCTGGCTGTCAATTCCCTCGGCGCTTCCCTTGTGAATTTTA
wsxfull.13.2.variant     2259 TGTTACGGTTCTGGCCATCAATTCAATTGGTGCTTCTGTTGCAAATTTTA

mu.wsx.ecd               2694 ACCTTACCTTCTCATGGCCCATGAGTAAAGTGAGTGCTGTGGAGTCACTC
wsxfull.13.2.variant     2309 ATTTAACCTTTTCATGGCCTATGAGCAAAGTAAATATCGTGCAGTCACTC

mu.wsx.ecd               2744 AGTGCTTATCCCCTGAGCAGCAGCTGTGTCATCCTTTCCTGGACACTGTC
wsxfull.13.2.variant     2359 AGTGCTTATCCTTTAAACAGCAGTTGTGTGATTGTTTCCTGGATACTATC

mu.wsx.ecd               2794 ACCTGATGATTATAGTCTGTTATATCTGGTTATTGAATGGAAGATCCTTA
wsxfull.13.2.variant     2409 ACCCAGTGATTACAAGCTAATGTATTTTATTATTGAGTGGAAAAATCTTA

mu.wsx.ecd               2844 ATGAAGATGATGGAATGAAGTGGCT
wsxfull.13.2.variant     2459 ATGAAGATGGTGAAATAAAATGGCTTAGAATCTCTTCATCTGTTAAGAAG

wsxfull.13.2.variant     2509 TATTATATCCATGATCATTTTATCCCCATTGAGAAGTACCAGTTCAGTCT

wsxfull.13.2.variant     2559 TTACCCAATATTTATGGAAGGAGTGGGAAAACCAAAGATAATTAATAGTT

wsxfull.13.2.variant     2609 TCACTCAAGATGATATTGAAAAACACCAGAGTGATGCAGGTTTATATGTA

wsxfull.13.2.variant     2659 ATTGTGCCAGTAATTATTTCCTCTTCCATCTTATTGCTTGGAACATTATT
```

Figure 5D

wsxfull.13.2.variant 2709 AATATCACACCAAAGAATGAAAAAGCTATTTTGGGAAGATGTTCCGAACC

wsxfull.13.2.variant 2759 CCAAGAATTGTTCCTGGGCACAAGGACTTAATTTTCAGAAGCCAGAAACG

wsxfull.13.2.variant 2809 TTTGAGCATCTTTTTATCAAGCATACAGCATCAGTGACATGTGGTCCTCT

wsxfull.13.2.variant 2859 TCTTTTGGAGCCTGAAACAATTTCAGAAGATATCAGTGTTGATACATCAT

wsxfull.13.2.variant 2909 GGAAAAATAAAGATGAGATGATGCCAACAACTGTGGTCTCTCTACTTTCA

wsxfull.13.2.variant 2959 ACAACAGATCTTGAAAAGGGTTCTGTTTGTATTAGTGACCAGTTCAACAG

wsxfull.13.2.variant 3009 TGTTAACTTCTCTGAGGCTGAGGGTACTGAGGTAACCTATGAGGACGAAA

wsxfull.13.2.variant 3059 GCCAGAGACAACCCTTTGTTAAATACGCCACGCTGATCAGCAACTCTAAA

wsxfull.13.2.variant 3109 CCAAGTGAAACTGGTGAAGAACAAGGGCTTATAAATAGTTCAGTCACCAA

wsxfull.13.2.variant 3159 GTGCTTCTCTAGCAAAAATTCTCCGTTGAAGGATTCTTTCTCTAATAGCT

wsxfull.13.2.variant 3209 CATGGGAGATAGAGGCCCAGGCATTTTTTATATTATCAGATCAGCATCCC

wsxfull.13.2.variant 3259 AACATAATTTCACCACACCTCACATTCTCAGAAGGATTGGATGAACTTTT

wsxfull.13.2.variant 3309 GAAATTGGAGGGAAATTTCCCTGAAGAAAATAATGATAAAAAGTCTATCT

wsxfull.13.2.variant 3359 ATTATTTAGGGGTCACCTCAATCAAAAAGAGAGAGAGTGGTGTGCTTTTG

wsxfull.13.2.variant 3409 ACTGACAAGTCAAGGGTATCGTGCCCATTCCCAGCCCCCTGTTTATTCAC

wsxfull.13.2.variant 3459 GGACATCAGAGTTCTCCAGGACAGTTGCTCACACTTTGTAGAAAATAATA

wsxfull.13.2.variant 3509 TCAACTTAGGAACTTCTAGTAAGAAGACTTTTGCATCTTACATGCCTCAA

wsxfull.13.2.variant 3559 TTCCAAACTTGTTCTACTCAGACTCATAAGATCATGGAAAACAAGATGTG

wsxfull.13.2.variant 3609 TGACCTAACTGTGTAATTTCACTGAAGAAACCTTCAGATTTGTGTTATAA

wsxfull.13.2.variant 3659 TGGGTAATATAAAGTGTAATAGATTATAGTTGTGGGTGGGAGAGAGAAAA

Figure 5E

wsxfull.13.2.variant 3709 G A A A C C A G A G T C A A A T T T G A A A A T A A T T G T T C C A A A T G A A T G T A G T C T G T

wsxfull.13.2.variant 3759 T T G T T C T C T C T T A G T A A C A T A G A C A A A A A A T T T G A G A A A G C C T T C A T A A G

wsxfull.13.2.variant 3809 C C T A C C A A T G T A G A C A C G C T C T T C T A T T T T A T T C C C A A G C T C T A G T G G G A

wsxfull.13.2.variant 3859 A G G T C C C T T G T T T C C A G C T A G A A A T A A G C C C A A C A G A C A O C A T C T T T T G T

wsxfull.13.2.variant 3909 G A G A T G T A A T T G T T T T T T C A G A G G G C G T G T T G T T T T A C C T C A A G T T T T T G

wsxfull.13.2.variant 3959 T T T T G T A C C A A C A C A C A C A C A C A C A C A C A T T C T T A A C A C A T G T C C T T G T G

wsxfull.13.2.variant 4009 T G T T T T G A G A G T A T A T T A T G T A T T T A T A T T T T G T G C T A T C A G A C T G T A G G

wsxfull.13.2.variant 4059 A T T T G A A G T A G G A C T T T C C T A A A T G T T T A A G A T A A A C A G A A T T C

Figure 5F

08/779457

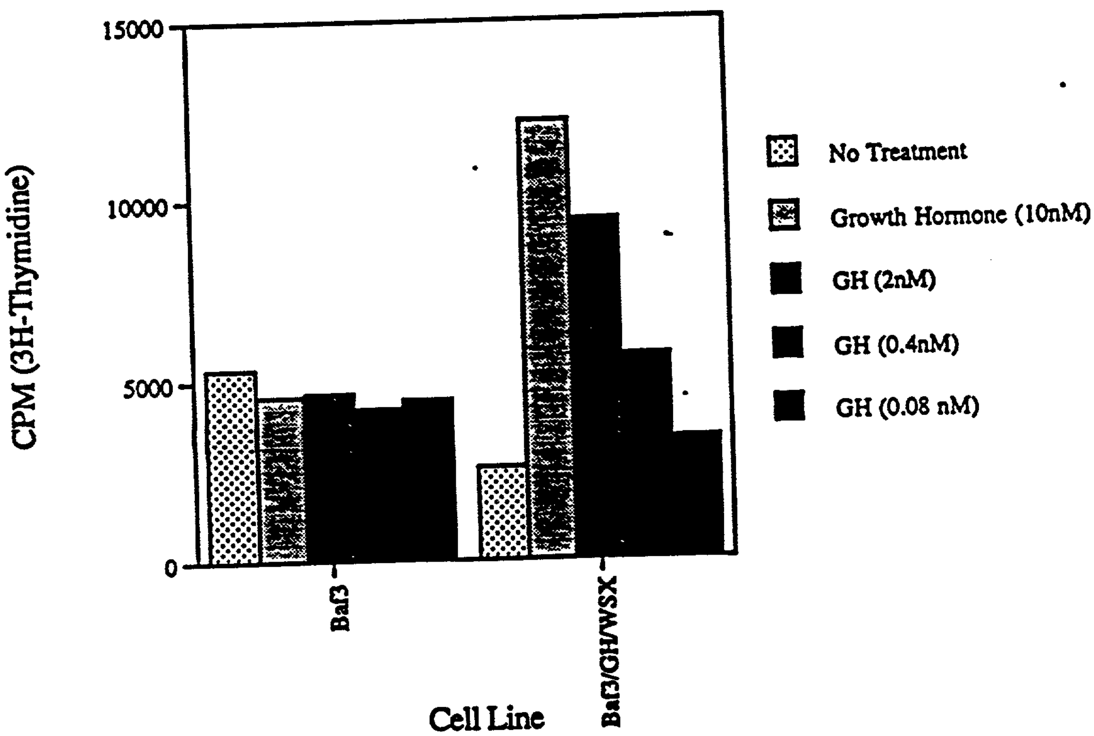


Figure 6

08/779457

Murine			
-213	Sense:	GGGTTAAGTTTCCCACCC	(SEQ ID NO:9)
	Antisense:	GGGTGGGAAACTTAACCC	(SEQ ID NO:10)
	Scrambled:	AGGATACAGTGGGATCCC	(SEQ ID NO:11)
-99	Sense:	GCCCGAGCACTCCTTTAA	(SEQ ID NO:12)
	Antisense:	TTAAAGGAGTGCTCCCGC	(SEQ ID NO:13)
	Scrambled:	GAGCGGCCCTGTTAGATA	(SEQ ID NO:14)
-20	Sense:	GTATACACCTCTGAAGAA	(SEQ ID NO:15)
	Antisense:	TTCTTCAGAGGTGTACAC	(SEQ ID NO:16)
	Scrambled:	ATGCGAGGCTACTTCTAT	(SEQ ID NO:17)
+84	Sense:	CTCTCCCTGGAAATTTAA	(SEQ ID NO:18)
	Antisense:	TTAAATTTCCAGGGAGAG	(SEQ ID NO:19)
	Scrambled:	ATTTGAAGGAGTTAAGCC	(SEQ ID NO:20)
+211	Sense:	AATTTAATTCAAGTGGTA	(SEQ ID NO:21)
	Antisense:	TACCAGTTGAATTAAATT	(SEQ ID NO:22)
	Scrambled:	GTATCACTTCATAATATA	(SEQ ID NO:23)
Human			
5L	Sense:	GATGGTCAGGGTGAACTG	(SEQ ID NO:24)
	Antisense:	CAGTTCACCCTGACCATC	(SEQ ID NO:25)
	Scrambled:	GAGGCGAATGTGCGGATT	(SEQ ID NO:26)
+85	Sense:	CTTAAATCTCCAAGGAGT	(SEQ ID NO:27)
	Antisense:	ACTCCTTGGAGATTTAAG	(SEQ ID NO:28)
	Scrambled:	AAGTCTTAAGCCAGACTT	(SEQ ID NO:29)
-47	Sense:	TCTAAGGCACATCCCAGC	(SEQ ID NO:30)
	Antisense:	GCTGGGATGTGCCTTAGA	(SEQ ID NO:31)
	Scrambled:	CGCAATGAATTGACCCCC	(SEQ ID NO:32)
-20	Sense:	TACTTCAGAGAAGTACAC	(SEQ ID NO:33)
	Antisense:	GTGTACTTCTCTGAAGTA	(SEQ ID NO:34)
	Scrambled:	GAATCACGGTAACTATCA	(SEQ ID NO:35)
+185	Sense:	CAGCTGTCTCATAATGTC	(SEQ ID NO:36)
	Antisense:	GACATTATGAGACAGCTG	(SEQ ID NO:37)
	Scrambled:	TTCGTCAAGCCATCTGAT	(SEQ ID NO:38)

Figure 7

08/779457

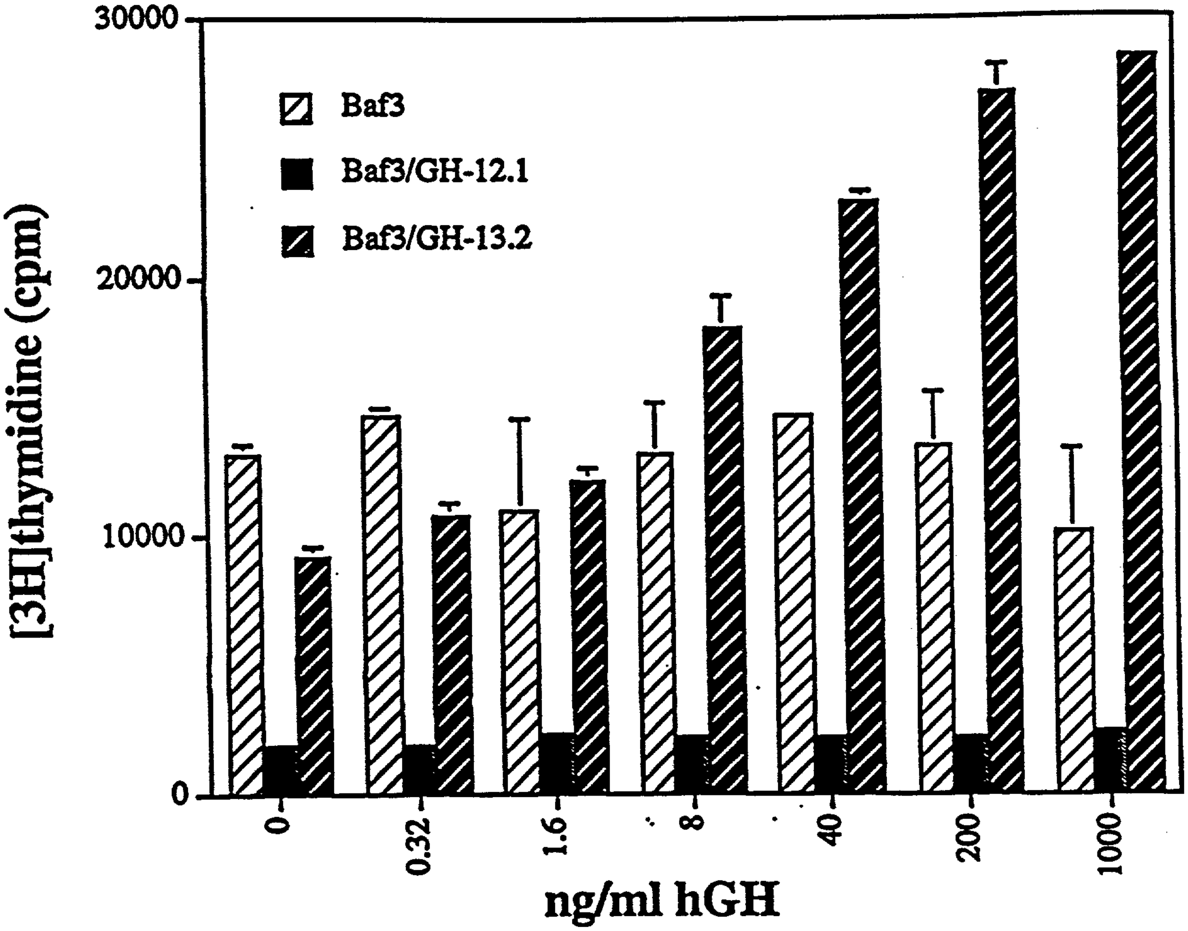


Figure 8

08/779457

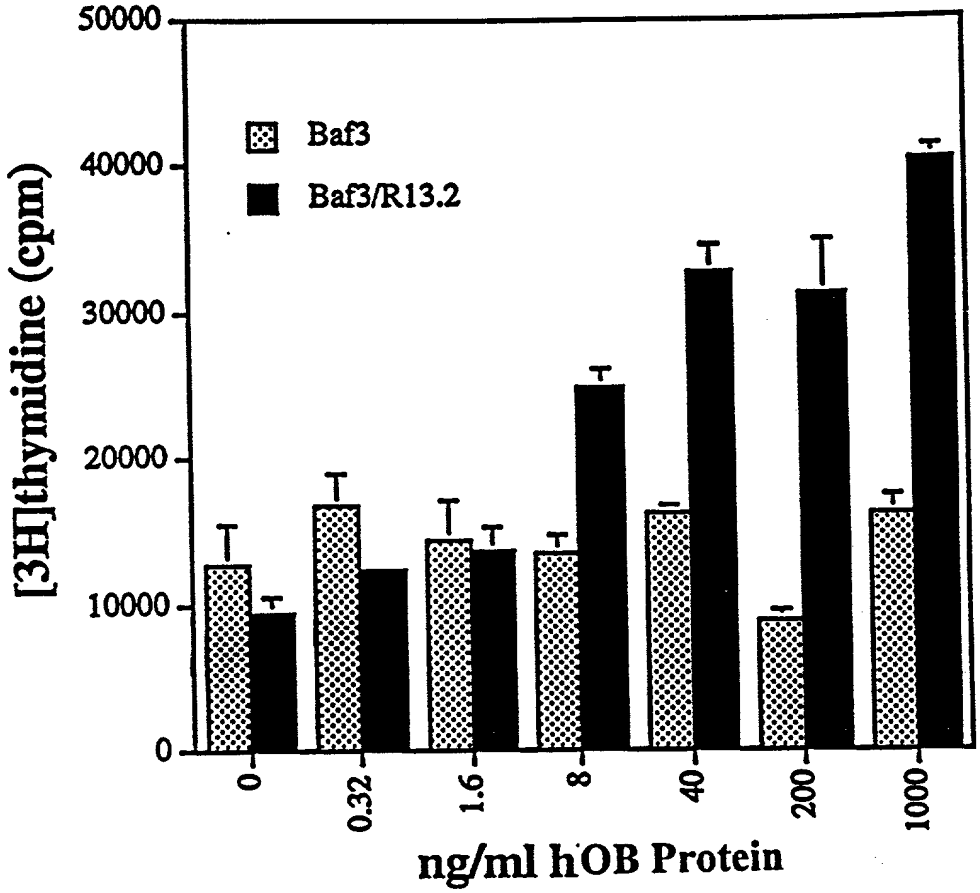


Figure 9

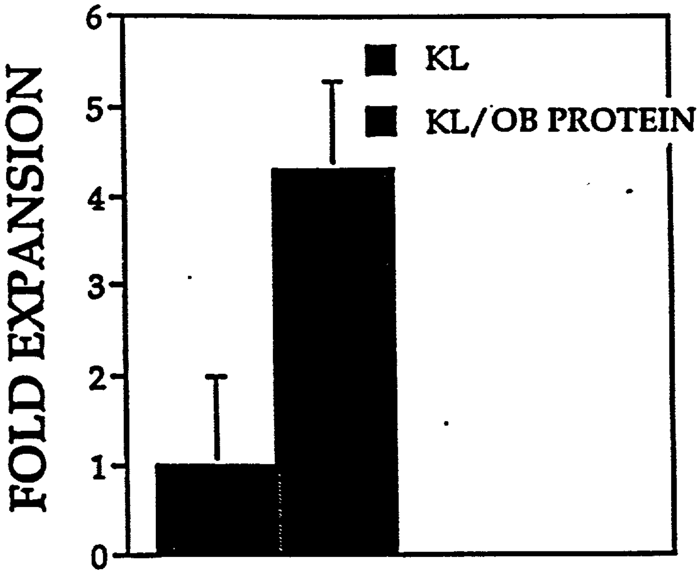


Figure 10A

OB flASK COLONY DATA

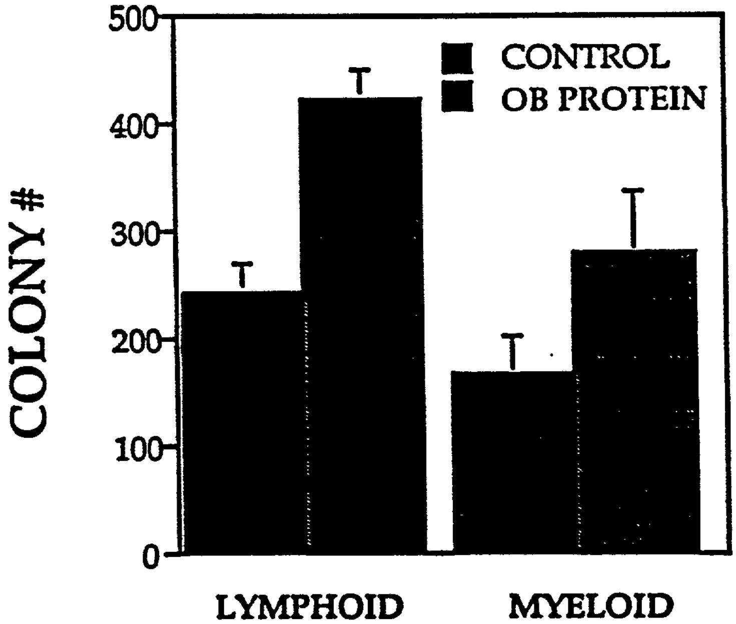


Figure 10B

08/779457

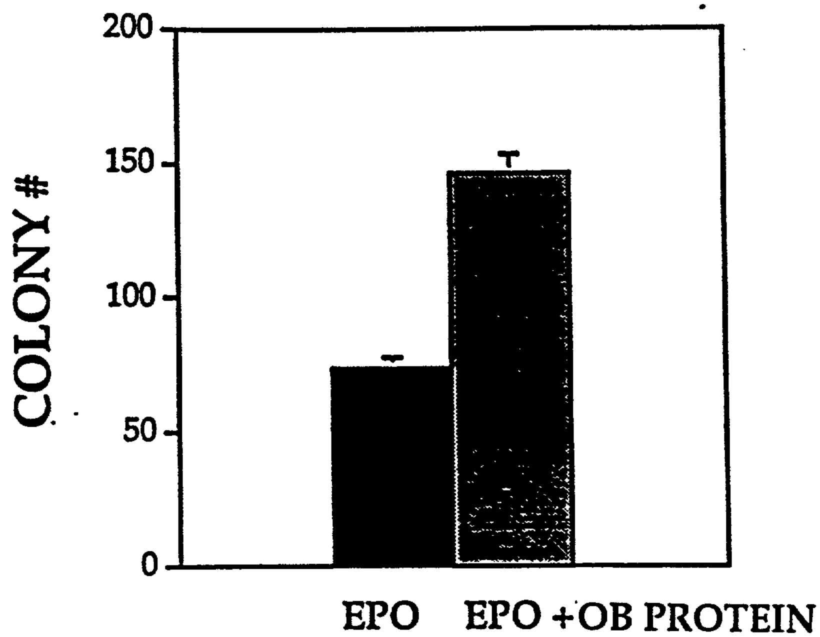


Figure 10C

46265154 . 013097

08/779457

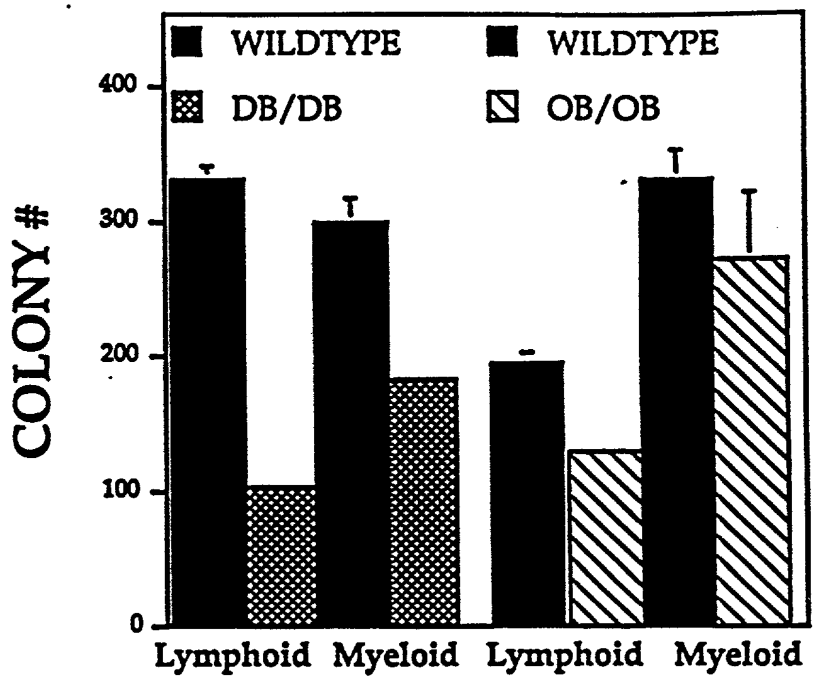


Figure 11

08/779457

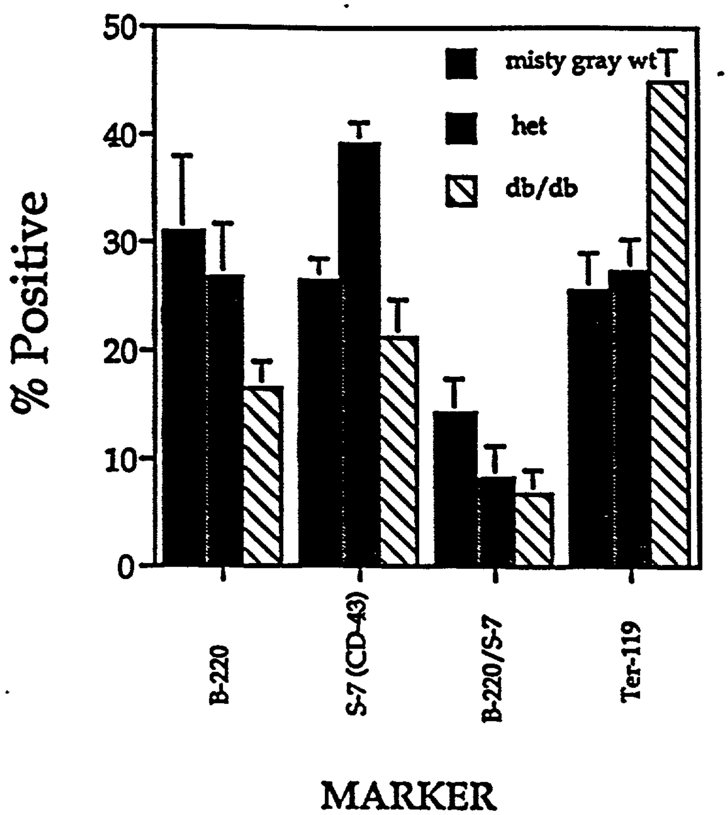
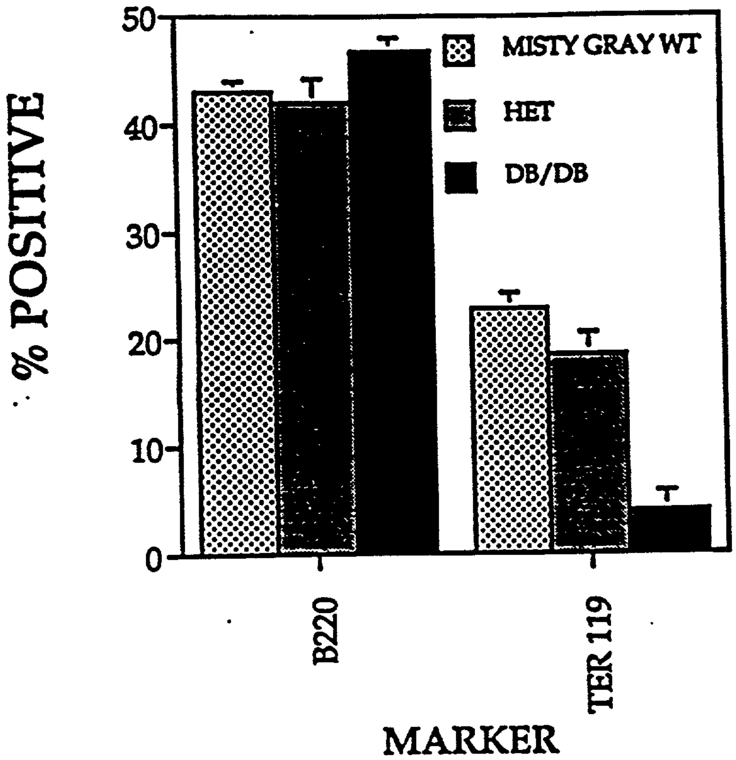


Figure 12A



% POSITIVE
50
40
30
20
10
0
MISTY GRAY WT
HET
DB/DB
B220
TER 119
MARKER

Figure 12B

08/779452

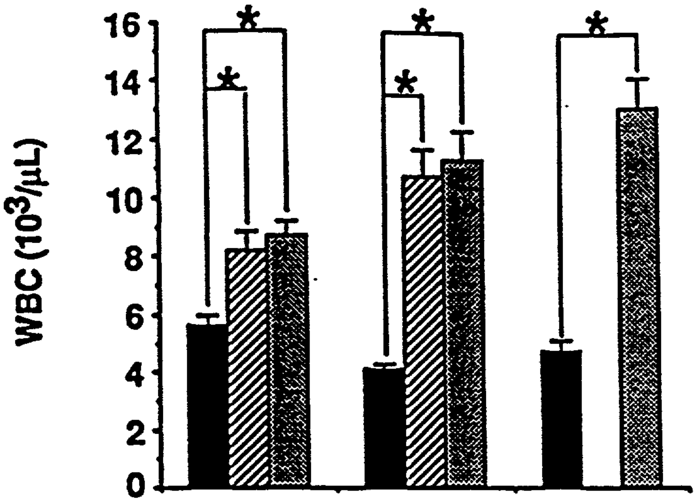


Figure 13A

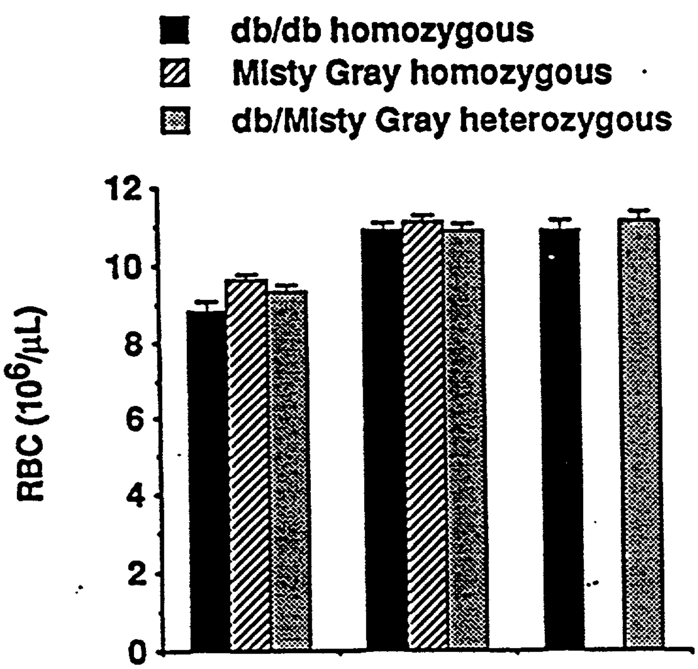


Figure 13B

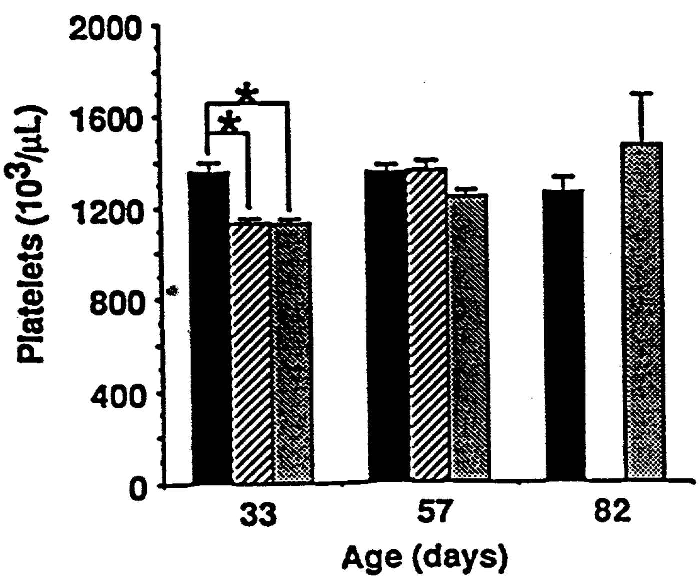


Figure 13C

08/779457

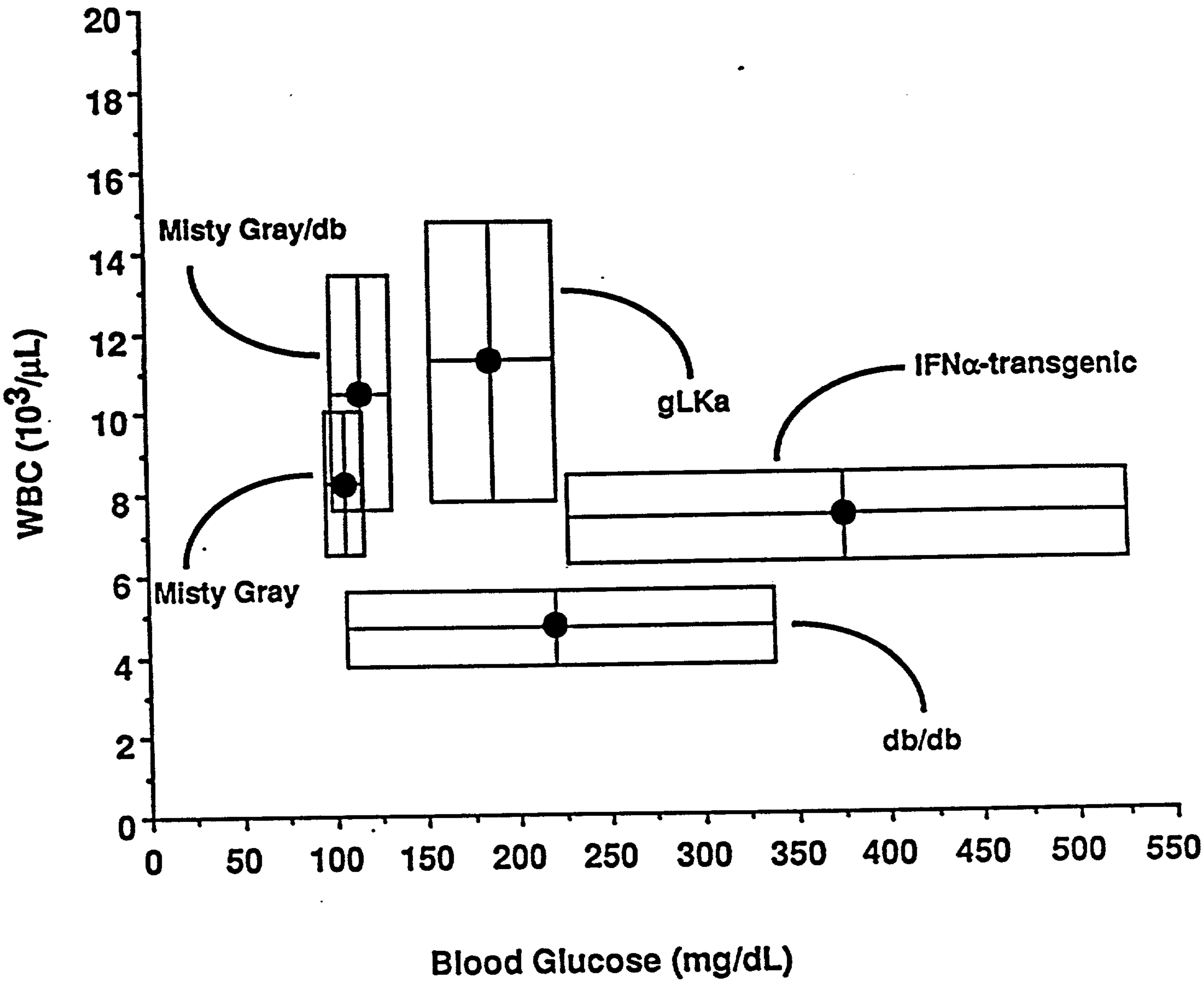


Figure 14

08/779457

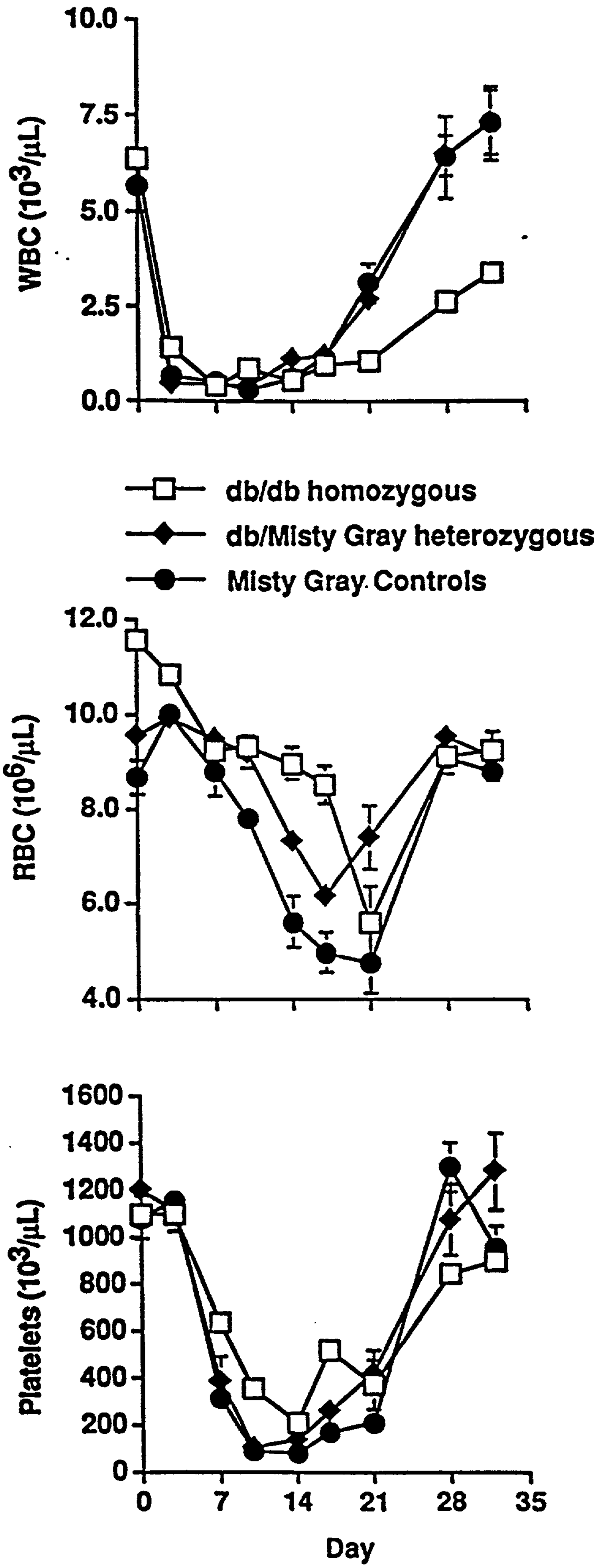


Figure 15A

Figure 15B

Figure 15C

08/779457

```
> sites: std
> length: 7127 (circular)

          aluI
         sstI
         sacI
         hgiJII
         hgiAI/aspHI
         ecl136II
         bsp1286
         bsiHKAI
         bmyI
         banII                          rmaI     tru9I                                               thaI
        taqI                            maeI     mseI                                                fnuDII/mvnI
                                        speI     aseI/asnI/vspI                                      bstUI
                                                                                      bslI           bsh1236I
                                                                                                    aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                              maeII
                sau96I                                  hinlI/acyI
                haeIII/palI    aciI                     ahaII/bsaHI
                asuI  apyI[dcm+]             aciI       aatII       maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                          maeII
     hinlI/acyI                                                                                     hinlI/acyI
     ahaII/bsaHI                                   rsaI                         rsaI                ahaII/bsaHI
     aatII                             bglI        csp6I             ndeI       csp6I               aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII                                                                                  nlaIII
            aciI                                                                                styI
          bglI  dsaV                                                                 maeII          ncoI
         sau96I bstNI           rsaI                                         rsaI    snaBI          dsaI hphI  aciI
         haeIII/palI            csp6I                                        csp6I   bsaAI          bsaJI  sfaNI
         asuI  apyI[dcm+]     bsrI  nlaIII
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

Figure 16A

08/779451

```
                                                                   maeII
                                                                   hinlI/acyI              nlaIV
                                                                   ahaII/bsaHI             hgiCI
        rsaI                         pleI                          aatII                   banI
        csp6I                aciI    hinfI            bsmAI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                           aluI
                                                                                           sstI
                                                                                           sacI
                                                                                           hgiJII
                                                                                           hgiAI/aspHI
                                                                                           ecl136II
                                                                                           bsp1286
                                                                                           bsiHKAI
                                                                                           bmyI
                                                                rsaI                       banII
                        maeIII      aciI       hgaI       aciI  csp6I      mnlI
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                     haeIII/palI
                                                                                     mcrI
                                                                                     eagI/xmaIII/eclXI
                                                                                     eaeI
                                                                                     cfrI
                                                                                     fnu4HI
                                                                                     aciI
                                                                                     thaI
                                                                                     fnuDII/mvnI
                 esp3I                                        sau96I           sacII/sstII
             scrFI                                                 avaII           nspBII
             mvaI bsmAI                                       asuI             kspI  scrFI
             ecoRII                                          nlaIV             dsaI  ncII
             dsaV                                                  scrFI        bglI bslI  mspI
             bstNI  hinlI/acyI                                     nciI     sau3AI mnlI bstUI
             apyI[dcm+]                                            mspI     mboI/ndeII[dam-] hpaII
         sau3AI gsuI/bpmI                                   mboII  hpaII    dpnI[dam+] bsaJI dsaV
         mboI/ndeII[dam-]                                   bpuAI  dsaV     dpnII[dam-] bsh1236I
         dpnI[dam+]  hgaI  fokI                             bbsI   cauII    alwI[dam-] aciI  cauII
         dpnII[dam-] ahaII/bsaHI              mnlI
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
              ^Begin RNA

                                                                                    fnu4HI
         tfiI                                                                       aciI
      aciI                                                        bstXI             thaI
     thaI hinfI                              aciI              sau96I   styI        fnuDII/mvnI  tru9I
     fnuDII/mvnI                    maeII rsaI         pleI scfI haeIII/palI        bstUI         mseI
     bstUI                         maeIII     csp6I   scfI hinfI   asuI    bsaJI     bsh1236I  aseI/asnI/vspI
     bsh1236I
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT
                                                                                 ^sp6 promoter
```

Figure 16B

08/779457

```
                                                                                                   sau96I
                                                                                                   avaII
                                                                                                   asuI
                                                                                                 scrFI
                                                                                                 mvaI
                                                                                                 ecoRII
                                                                                                 dsaV
                                                                                                 bstNI
                                              maeIII                                             apyI[dcm+]
                                             hphI    scfI        fokI                       bslI bsaJI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG
                                                   ^sp6 RNA start

                       ppu10I                    tfiI               sau96I
     mnlI          taqI nsiI/avaIII              hinfI              haeIII/palI                 bsp1286
     bsaJI        claI/bsp106       nlaIV      aciI                 asuI                  aluI  bmyI fokI
901 ACCTCGGTTC TATCGATATG CATTGGGGAA CCCTGTGCGG ATTCTTGTGG CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT GTGCCCATCC AAAAAGTCCA
    TGGAGCCAAG ATAGCTATAC GTAACCCCTT GGGACACGCC TAAGAACACC GAAACCGGGA TAGAAAAGAT ACAGGTTCGA CACGGGTAGG TTTTTCAGGT
  1                    Met HisTrpGlyT hrLeuCysGl yPheLeuTrp LeuTrpProT yrLeuPheTy rValGlnAla ValProIleG lnLysValGln
     ^cloning linker   ^human OB start

                                                 sau3AI
                                                 mboI/ndeII[dam-]
                                                 dpnI[dam+]
                                              scrFI
                                              mvaI                                                        mspI
                                              ecoRII                                                 hpaII
                                              dsaV                                                  cfr10I
                                              bstNI                                                     bsaWI
                                              apyI[dcm+]                                                ageI
                                            hphI   dpnII[dam-]                            mnlI         hphI
                         mnlI        munI  maeIII alwI[dam-]                          bsmAI          maeIII
1001 AGATGACACC AAAACCCTCA TCAAGACAAT TGTCACCAGG ATCAATGACA TTTCACACAC GCAGTCAGTC TCCTCCAAAC AGAAAGTCAC CGGTTTGGAC
     TCTACTGTGG TTTTGGGAGT AGTTCTGTTA ACAGTGGTCC TAGTTACTGT AAAGTGTGTG CGTCAGTCAG AGGAGGTTTG TCTTTCAGTG GCCAAACCTG
  29  AspAspThr LysThrLeuI leLysThrIl eValThrArg IleAsnAspI leSerHisTh rGlnSerVal SerSerLysG lnLysValTh rGlyLeuAsp

        hgiJII
        bsp1286
        bmyI
        banII                                                                          hphI
      scrFI                                                                      mnlI
      mvaI nlaIV                                    alwNI                          sau3AI   bsrI                sau3AI
      ecoRII                                   pflMI                          mboI/ndeII[dam-]             mboI/ndeII[dam-]
      dsaV                                     bslI                           dpnI[dam+]                   dpnI[dam+]
      bstNI                                       sau96I                          dpnII[dam-]                  dpnII[dam-]
      bsaJI                                       avaII                           alwI[dam-]                   alwI[dam-]
      apyI[dcm+]       fokI                       asuI       bsrI    accI        bstYI/xhoII              maeII
1101 TTCATTCCTG GGCTCCACCC CATCCTGACC TTATCCAAGA TGGACCAGAC ACTGGCAGTC TACCAACAGA TCCTCACCAG TATGCCTTCC AGAAACGTGA
     AAGTAAGGAC CCGAGGTGGG GTAGGACTGG AATAGGTTCT ACCTGGTCTG TGACCGTCAG ATGGTTGTCT AGGAGTGGTC ATACGGAAGG TCTTTGCACT
  62 PheIleProG lyLeuHisPr oIleLeuThr LeuSerLysM etAspGlnTh rLeuAlaVal TyrGlnGlnI leLeuThrSe rMetProSer ArgAsnValIle
```

Figure 16C

```
                                                                                           haeIII/palI
                                                  pmlI                                    sau96I        styI
                                        sau3AI    eco72I                                  asuI         gsuI/bpmI
                                       bstYI/xhoII                                     scrFI   scrFI
                       gsuI/bpmI   scrFI          bsaAI                                mvaI          mvaI bsmAI
                      scrFI  ncII mboI/ndeII[dam-]                               ecoRII ecoRII
                      mvaI        mspI dpnI[dam+]                                      dsaV    dsaV
                      ecoRII hpaII    mboII maeII                                 bstNI         bstNI    bsaJI
                      dsaV   dsaV dpnII[dam-]                      fnu4HI         bsaJI bstXI   apyI[dcm+]
                      bstNI     mnlI  alwI[dam-] bbrPI     haeIII/palI     bbvI        apyI[dcm+] haeI    bsaI
              bstXI   apyI[dcm+]  cauII  mboII[dam-]      haeI      ddeI  aluI        bsaJI   bsrI haeIII/palI
1201 TCCAAATATC CAACGACCTG GAGAACCTCC GGGATCTTCT TCACGTGCTG GCCTTCTCTA AGAGCTGCCA CTTGCCCTGG GCCAGTGGCC TGGAGACCTT
     AGGTTTATAG GTTGCTGGAC CTCTTGGAGG CCCTAGAAGA AGTGCACGAC CGGAAGAGAT TCTCGACGGT GAACGGGACC CGGTCACCGG ACCTCTGGAA
  96  GlnIleSe rAsnAspLeu GluAsnLeuA rgAspLeuLe uHisValLeu AlaPheSerL ysSerCysHi sLeuProTrp AlaSerGlyL euGluThrLeu

                                                                                                            sau96I
           scrFI           eco57I                                                                           avaII
           mvaI         scrFI
           ecoRII mvaI   aluI                                 ddeI      scfI                             aluI
           dsaV   ecoRII                              bslI        pstI         scfI                pvuII
           bstNI        dsaV                              sau96I        bsgI          pstI     nlaIII     nspBII
           bsaJI        bstNI hindIII         xcmI              haeIII/palI  fnu4HI     bsmAI       nspI       fnu4HI
           apyI[dcm+]   apyI[dcm+]            bstXI mnlI        asuI         bbvI      bsaI bsgI    nspHI      bbvI  asuI
1301 GGACAGCCTG GGGGGTGTCC TGGAAGCTTC AGGCTACTCC ACAGAGGTGG TGGCCCTGAG CAGGCTGCAG GGGTCTCTGC AGGACATGCT GTGGCAGCTG
     CCTGTCGGAC CCCCCACAGG ACCTTCGAAG TCCGATGAGG TGTCTCCACC ACCGGGACTC GTCCGACGTC CCCAGAGACG TCCTGTACGA CACCGTCGAC
 129  AspSerLeu GlyGlyValL euGluAlaSe rGlyTyrSer ThrGluValV alAlaLeuSe rArgLeuGln GlySerLeuG lnAspMetLe uTrpGlnLeu

              scrFI                                                                  eam1105I
              mvaI                                                                        sau96I
              ecoRII                                                          scrFI
              dsaV                                                            mvaI    avaII
              bstNI                                                                  ecoRII
              bslI                                                                   dsaV
              bsaJI          hphI                    nlaIII                          bstNI   asuI          mboII  mboII
        ddeI  apyI[dcm+]    maeIII                 nspI          bsp1286             bsaJI  nlaIV        bpuAI  earI/ksp632I
       mnlI  bsaJI . . aciI bstEII                 nspHI         bmyI alwNI          apyI[dcm+]          bbsI mnlI
1401 GACCTCAGCC CTGGGTGCGG GGTCACCGAC AAAACTCACA CATGCCCACC GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC
     CTGGAGTCGG GACCCACGCC CCAGTGGCTG TTTTGAGTGT GTACGGGTGG CACGGGTCGT GGACTTGAGG ACCCCCCTGG CAGTCAGAAG GAGAAGGGGG
 162 AspLeuSerP roGlyCysGl yValThrAsp LysThrHisT hrCysProPr oCysProAla ProGluLeuL euGlyGlyPr oSerValPhe LeuPheProPro
                         ^insertion of a gly
                                  ^START OF HUMAN IgG1 CH2CH3
```

Figure 16D

08/779457

```
                                                  sau96I
                                                  nlaIV
                                               mspI
                                               hpaII
                                              scrFI
                                              nciI
                                              dsaV
                                         sau3AI    avaII
                                         mboI/ndeII[dam-]              nlaIII
                                     nlaIII    cauII         mnlI    nspI                          drdI    mnlI                  maeII
                                    rcaI dpnI[dam+]        ddeI      nspHI                      mboII   ddeI                   rsaI
              styI                  bspHI[dam-]     asuI eco81I maeIII                           bpuAI  eco81I                   csp6I
              bsaJI               mnlI     dpnII[dam-]    bsu36I/mstII/sauI          maeII       bbsI   bsu36I/mstII/sauI bsrI bsaAI
1501 CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA
     GTTTTGGGTT CCTGTGGGAG TACTAGAGGG CCTGGGGACT CCAGTGTACG CACCACCACC TGCACTCGGT GCTTCTGGGA CTCCAGTTCA AGTTGACCAT
196   LysProLy sAspThrLeu MetIleSerA rgThrProGl uValThrCys ValValValA spValSerHi sGluAspPro GluValLysP heAsnTrpTyr

                                                       aciI
                                                      thaI
                                                      fnuDII/mvnI
                                                      bstUI
                                                      bsh1236I
                                                     sacII/sstII
                                                     nspBII
                                                     kspI                                                                   scrFI
                                                     dsaI                               rsaI                                mvaI
                                                     bsaJI                              csp6I                               ecoRII
                                                     aciI                 rsaI        maeII                   hphI          dsaV
                         mnlI                       fnu4HI   mnlI         csp6I       bsaAI          hgaI mnlI      econI  bstNI
                                                                                                                    bslI   apyI[dcm+]
1601 CGTGGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTCACCGT CCTGCACCAG
     GCACCTGCCG CACCTCCACG TATTACGGTT CTGTTTCGGC GCCCTCCTCG TCATGTTGTC GTGCATGGCA CACCAGTCGC AGGAGTGGCA GGACGTGGTC
229   ValAspGly ValGluValH isAsnAlaLy sThrLysPro ArgGluGluG lnTyrAsnSe rThrTyrArg ValValSerV alLeuThrVa lLeuHisGln

                                                                                                            fnu4HI
                                rsaI        bsmAI                                                           bbvI   avaI
        bsrI                    csp6I       bsaI              mnlI            taqI
1701 GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG
     CTGACCGACT TACCGTTCCT CATGTTCACG TTCCAGAGGT TGTTTCGGGA GGGTCGGGGG TAGCTCTTTT GGTAGAGGTT TCGGTTTCCC GTCGGGGCTC
262 AspTrpLeuA snGlyLysGl uTyrLysCys LysValSerA snLysAlaLe uProAlaPro IleGluLysT hrIleSerLy sAlaLysGly GlnProArgGlu
```

Figure 16E

08/779457

```
                                  scrFI
                                  nciI
                                  mspI
                                  hpaII
                                  dsaV
                                  cauII
                                 xmaI/pspAI
                                 smaI
                                 scrFI                   scrFI          scrFI
                                 nciI                    mvaI                mvaI
                                 dsaV                    ecoRII         ecoRII
                                 cauII                   dsaV           dsaV
                rsaI           fokI                      bstNI                bstNI                            dsaI
                csp6I       bslI bsaJI mboII             apyI[dcm+]           apyI[dcm+]                       bslI
              bsp1407I    bslI  avaI  earI/ksp632I     sexAI          bspMI                                    bsaJI
1801 AACCACAGGT GTACACCCTG CCCCCATCCC GGGAAGAGAT GACCAAGAAC CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC
     TTGGTGTCCA CATGTGGGAC GGGGGTAGGG CCCTTCTCTA CTGGTTCTTG GTCCAGTCGG ACTGGACGGA CCAGTTTCCG AAGATAGGGT CGCTGTAGCG
296   ProGlnVa lTyrThrLeu ProProSerA rgGluGluMe tThrLysAsn GlnValSerL euThrCysLe uValLysGly PheTyrProS erAspIleAla

                                                                                                               dsaI
                                mspI
                                hpaII
                            fnu4HI                                      pleI                  mnlI        hphI
                            bbvI                          mnlI          hinfI     nlaIV  mboII   scfI     aluI  bsaJI
1901 CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC
     GCACCTCACC CTCTCGTTAC CCGTCGGCCT CTTGTTGATG TTCTGGTGCG GAGGGCACGA CCTGAGGCTG CCGAGGAAGA AGGAGATGTC GTTCGAGTGG
329  ValGluTrp GluSerAsnG lyGlnProGl uAsnAsnTyr LysThrThrP roProValLe uAspSerAsp GlySerPheP heLeuTyrSe rLysLeuThr

                                                                                                                scrFI
                                                                                                                nciI
                                      mboII                                                                     mspI
                                     bpuAI                  nlaIII                                              hpaII
                                  maeII                 ppu10I                                  sapI            dsaV
                      fnu4HI    xmnI bbsI               nsiI/avaIII                             mboII mnlI   bsmAI
               bspMI  bbvI      asp700      nlaIII     sfaNI   mnlI                             earI/ksp632I bslI cauII
2001 GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC
     CACCTGTTCT CGTCCACCGT CGTCCCCTTG CAGAAGAGTA CGAGGCACTA CGTACTCCGA GACGTGTTGG TGATGTGCGT CTTCTCGGAG AGGGACAGAG
362  ValAspLysS erArgTrpGl nGlnGlyAsn ValPheSerC ysSerValMe tHisGluAla LeuHisAsnH isTyrThrGl nLysSerLeu SerLeuSerPro

                                                                                        sau96I
                                                                                     nlaIII
                                                                               fnu4HI   haeIII/palI
                                  taqI                                         bglI styI
                                 salI                       taqI              sfiI  ncoI
                               pleI     scfI            pleI     scfI        eaeI   dsaI
                            rmaI hincII/hindII        rmaI  salI  pstI       cfrI   bsaJI                        aluI
                       sau96I  hinfI    pstI        xbaI    hincII/hindII                                       fnu4HI
                       haeIII/palI      bsgI    aluI maeI   accI  bsgI  aluI haeIII/palI                        bbvI
                       asuI maeI accI bspMI  hindIII    hinfI  bspMI   hindIII   aciI  asuI
2101 CGGGTAAATG AGTGCGACGG CCCTAGAGTC GACCTGCAGA AGCTTCTAGA GTCGACCTGC AGAAGCTTGG CCGCCATGGC CCAACTTGTT TATTGCAGCT
     GCCCATTTAC TCACGCTGCC GGGATCTCAG CTGGACGTCT TCGAAGATCT CAGCTGGACG TCTTCGAACC GGCGGTACCG GGTTGAACAA ATAACGTCGA
396   GlyLys
                                                                                                 ^sv40 early poly A
```

Figure 16F

08/779457

08/779457

```
                                                                                                  rmaI
          maeIII                    sfaNI    apoI                                         bsmI maeI
2201 TATAATGGTT ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT
     ATATTACCAA TGTTTATTTC GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA

                     sau3AI
                     mboI/ndeII[dam-]
                     dpnI[dam+]
                     dpnII[dam-]
                    pvuI/bspCI
                    mcrI
                   taqI[dam-]  tru9I
                  claI/bsp106[dam-]                 haeIII/palI
                 sau3AI       mseI                 haeI                                   rsaI
                 mboI/ndeII[dam-]      fnu4HI  styI                                        csp6I
                 dpnI[dam+] xmnI       bbvI    ncoI                                       nlaIV
                 dpnII[dam-]  aseI/asnI/vspI  hinPI  dsaI                                 kpnI
                                       hhaI/cfoI  bsaJI                                   hgiCI
                                                  nlaIII                                  banI
                                                                                          asp718    mnlI
                                                                      mnlI      mnlI     acc65I  ddeI  aciI
          nlaIII  alwI[dam-]  asp700
2301 CTTATCATGT CTGGATCGAT CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA
     GAATAGTACA GACCTAGCTA GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT
                                ^sv40 origin

                                                     nlaIV
                                                   scrFI                      sfaNI                scrFI
                                                   mvaI                          ppu10I                 mvaI
                                                   ecoRII                    nsiI/avaIII           ecoRII
                                                   dsaV                     nlaIII                 dsaV
                                                   bstNI                        sphI                    bstNI
          aluI                                     apyI[dcm+]                   nspI                    apyI[dcm+]
          pvuII                                   bsaJI                         nspHI                  sexAI
          nspBII
2401 AGAACCAGCT GTGGAATGTG TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC
     TCTTGGTCGA CACCTTACAC ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG

                  nlaIV
                 scrFI
                 mvaI                                ppu10I
                 ecoRII                             nsiI/avaIII
                 dsaV                              nlaIII
                 bstNI                                sphI
                 apyI[dcm+]                           nspI  sfaNI                   aciI      aciI  fokI
                bsaJI                                 nspHI
2501 CAGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC
     GTCCACACCT TTCAGGGGTC CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG

                                                                                fnu4HI
                                                                                bglI
                                                 nlaIII                        sfiI
                                                 styI                          haeIII/palI
                                                 ncoI                        mnlI    mnlI         ddeI
                 bsrI                         bslI dsaI                     haeIII/palI bsaJI mnlI   aluI
     aciI          aciI         aciI          aciI bsaJI                  mnlI bsaJI aciI    haeIII/palI
2601 CCGCCCCTAA CTCCGCCCAG TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT
     GGCGGGGATT GAGGCGGGTC AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA
```

Figure 16G

```
                                                                                                                   scrFI
                                                                                                                   mvaI
                                                                                                              sau96I
                                                                                                              nlaIV
                                                                                                              avaII    sau96I
                                                                                                           thaI     ecoRII
                                                                                                           fnuDII/mvnI
                                  styI                                                                     bstUI    dsaV
                                  bsaJI                        sfuI                                        bsh1236I   avaII
                                  blnI                         bstBI                                       hinPI    bstNI
                               haeIII/palI                     bsiCI                                      hhaI/cfoI   asuI
                              stuI rmaI                        asuII                                      fnu4HI asuI apyI[dcm+]
                mnlI          haeI maeI                     tru9I                                         aciI aciI  bsaJI
             mnlI            mnlI avrII            aluI     mseI taqI       sfaNI
2701 TCCAGAAGTA GTGAGGAGGC TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAATTC GAACACGCAG ATGCAGTCGG GGCGGCGCGG TCCCAGGTCC
     AGGTCTTCAT CACTCCTCCG AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTAAG CTTGTGCGTC TACGTCAGCC CCGCCGCGCC AGGGTCCAGG
                                                      ^start pUC118
                                                               ^TK promoter

                                                                                                                  sau3AI
                                                                                                                  mboI/ndeII[dam-]
                                                                                                                  dpnI[dam+]
                                                                                                            sau3AI
                    thaI                                                                                    mboI/ndeII[dam-]
                    fnuDII/mvnI                                                                             dpnI[dam+]
                    bstUI                                                                                   bstYI/xhoII
                   mluI                          fnu4HI                                                     bglII  dpnII[dam-]
                   aflIII                        bbvI                                                       hincII/hindII aciI dpnII[dam-] bsmAI
               hphI bsh1236I  mnlI               scfI                    tru9I
           tru9I  hgaI       haeIII/palI         pstI
           mseI maeIII      haeI   taqI          bsgI        aciI mseI  hgaI          fnu4HI      bclI[dam-]
2801 ACTTCGCATA TTAAGGTGAC GCGTGTGGCC TCGAACACCG AGCGACCCTG CAGCGACCCG CTTAACAGCG TCAACAGCGT GCCGCAGATC TGATCAAGAG
     TGAAGCGTAT AATTCCACTG CGCACACCGG AGCTTGTGGC TCGCTGGGAC GTCGCTGGGC GAATTGTCGC AGTTGTCGCA CGGCGTCTAG ACTAGTTCTC
                                                                  tn5 neomycin phosphotransferase gene.  ^

                                                                  aciI
                                                                  fnu4HI
           sau3AI                                                haeIII/palI
           mboI/ndeII[dam-]                                      mcrI
           dpnI[dam+]                                            eagI/xmaIII/eclXI
           dpnII[dam-]                                           eaeI
        mnlI                                                     cfrI
      mamI[dam-]                                                 mspI                                     bsp1286
      bsaBI[dam-]                                                                                          bmyI
     fokI  alwI[dam-]   nlaIII                     bspMI         hpaII            mnlI              bsrI
2901 ACAGGATGAG GATCGTTTCG CATGATTGAA CAAGATGGAT TGCACGCAGG TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC
     TGTCCTACTC CTAGCAAAGC GTACTAACTT GTTCTACCTA ACGTGCGTCC AAGAGGCCGG CGAACCCACC TCTCCGATAA GCCGATACTG ACCCGTGTTG
```

08/779457

Figure 16H

```
                                                          hinPI
                                                          hhaI/cfoI
                                                         nlaIV
                                                         narI scrFI                             bsp1286
                                                         kasI ncII                              bmyI
                                                         hinII/acyI                            nlaIV
                                                         hgiCI mspI                            hgiCI
                                                         haeII hpaII                           banI
                            aciI                         banI dsaV                           mspI              scfI
              fnu4HI       fnu4HI       mspI        hinPI ahaII/bsaHI                        hpaII             pstI
              bbvI     sfaNI   bslI     hpaII       hhaI/cfoI  cauII                drdI    bsaWI             bsgI
3001 AGACAATCGG CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG CGCCCGGTTC TTTTTGTCAA GACCGACCTG TCCGGTGCCC TGAATGAACT
     TCTGTTAGCC GACGAGACTA CGGCGGCACA AGGCCGACAG TCGCGTCCCC GCGGGCCAAG AAAAACAGTT CTGGCTGGAC AGGCCACGGG ACTTACTTGA

                                                                     hgiAI/aspHI
                     fnu4HI                                          bsp1286
                    thaI                                       aluI
                    fnuDII/mvnI                               pvuII
                    bstUI                                    fnu4HI
                    bsh1236I          haeIII/palI            bbvI   bsiHKAI
                   hinPI            mscI/balI              hinPI    bmyI
                fnu4HI              haeI                   hhaI/cfoI       maeII     eco57I
                bbvI aciI           eaeI                 mstI nspBII      tth111I/aspI                 fnu4HI
            mnlI   hhaI/cfoI        cfrI                 aviII/fspI     taqI     maeIII   aciI      bsrI bbvI
3101 GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GCCGTTCCTT GCGCAGCTGT GCTCGACGTT GTCACTGAAG CGGGAAGGGA CTGGCTGCTA
     CGTCCTGCTC CGTCGCGCCG ATAGCACCGA CCGGTGCTGC CCGCAAGGAA CGCGTCGACA CGAGCTGCAA CAGTGACTTC GCCCTTCCCT GACCGACGAT

                  scrFI
                  ncII      sau3AI
                  mspI      mboI/ndeII[dam-]                                                  fnu4HI        sau3AI
                  hpaII     dpnI[dam+]                                                     fnu4HI           mboI/ndeII[dam-]
                  dsaV      dpnII[dam-]                                                    aciI             dpnI[dam+]
                  cauII    bstYI/xhoII                                                   fnu4HI             dpnII[dam-]
                  bsaJI    alwI[dam-]       hphI                        nlaIII  sfaNI    aciI  bbvI         alwI[dam-]
3201 TTGGGCGAAG TGCCGGGGCA GGATCTCCTG TCATCTCACC TTGCTCCTGC CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACGCTTG
     AACCCGCTTC ACGGCCCCGT CCTAGAGGAC AGTAGAGTGG AACGAGGACG GCTCTTTCAT AGGTAGTACC GACTACGTTA CGCCGCCGAC GTATGCGAAC

                                                                                                     sau3AI
                                                                                                     mboI/ndeII[dam-]
                                                                rsaI                             fokI
                                                                csp6I                        sau3AI   dpnI[dam+]
                                                             bsaAI                           mboI/ndeII[dam-]   sapI
                                                         hgiAI/aspHI                         dpnI[dam+]         mboII
                                                         bsp1286               mspI          dpnII[dam-]        earI/ksp632I
       mspI                                     taqI     bsiHKAI               hpaII
       hpaII bspMI      taqI                 sfaNI       bmyI maeII    fokI   cfr10I      taqI[dam-]  dpnII[dam-]
3301 ATCCGGCTAC CTGCCCATTC GACCACCAAG CGAAACATCG CATCGAGCGA GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA
     TAGGCCGATG GACGGGTAAG CTGGTGGTTC GCTTTGTAGC GTAGCTCGCT CGTGCATGAG CCTACCTTCG GCCAGAACAG CTAGTCCTAC TAGACCTGCT
```

08/779457

Figure 16I

```
                                                            sphI
                                                            nspI
                                                            nspHI
                                                         hinPI
                      hinPI                              hhaI/cfoI
                     thaI                               thaI                  sau3AI
                     fnuDII/mvnI           scrFI  fnuDII/mvnI           mboI/ndeII[dam-]
                     bstUI                 mvaI         bstUI                 dpnI[dam+]      styI
                hgiJII                     ecoRII bsh1236I              dpnII[dam-]     ncoI
                bsp1286                    dsaV  hinPI nlaIII          bstYI/xhoII      dsaI
                bmyI bsh1236I              bstNI       hhaI/cfoI             alwI[dam-]       bsaJI  sfaNI
        sfaNI   banII hhaI/cfoI            apyI[dcm+]  bssHII             mnlI         maeIII nlaIII
3401 AGAGCATCAG GGGCTCGCGC CAGCCGAACT GTTCGCCAGG CTCAAGGCGC GCATGCCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG
     TCTCGTAGTC CCCGAGCGCG GTCGGCTTGA CAAGCGGTCC GAGTTCCGCG CGTACGGGCT GCCGCTCCTA GAGCAGCACT GGGTACCGCT ACGGACGAAC

                                                            mspI               bslI
                                                            hpaII          sau96I
                              aciI                        naeI             avaII
                             fnu4HI                       cfr10I           asuI
                            haeIII/palI                  haeIII/palI      rsrII/cspI
                           eaeI           tfiI          eaeI               cpoI
              nlaIII       cfrI           hinfI  taqI   cfrI              aciI aciI
3501 CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG
     GGCTTATAGT ACCACCTTTT ACCGGCGAAA AGACCTAAGT AGCTGACACC GGCCGACCCA CACCGCCTGG CGATAGTCCT GTATCGCAAC CGATGGGCAC

              sapI                                                                      hinPI
              mboII      fnu4HI                                        bsrBI            hhaI/cfoI
              earI/ksp632I                                             aciI    tfiI  fnu4HI
           eco57I aluI   aciI             aciI   mnlI                fnu4HI    hinfI bbvI sfaNI
3601 ATATTGCTGA AGAGCTTGGC GGCGAATGGG CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT
     TATAACGACT TCTCGAACCG CCGCTTACCC GACTGGCGAA GGAGCACGAA ATGCCATAGC GGCGAGGGCT AAGCGTCGCG TAGCGGAAGA TAGCGGAAGA

                                       taqI
                                      sfuI
                       aciI           bstBI                 hinlI/acyI                    tfiI       aciI
                   ddeI     pleI      bsiCI                 hgaI    bspMI                 hinfI     fnu4HI
              mboII  bsrBI  hinfI     asuII                 ahaII/bsaHI                 taqI      aciI
3701 TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC GACGCCCAAC CTGCCATCAC GAGATTTCGA TTCCACCGCC GCCTTCTATG
     ACTGCTCAAG AAGACTCGCC CTGAGACCCC AAGCTTTACT GGCTGGTTCG CTGCGGGTTG GACGGTAGTG CTCTAAAGCT AAGGTGGCGG CGGAAGATAC
```

Figure 16J

08/779457

08/779457

```
                                                                                                    scrFI
                                                                                                    nciI
                                                                                                    mspI
                                                                                                    hpaII
                                                                                                    dsaV
                                                                                                    cauII
                                                                                                    bslI
                                    hinlI/acyI                 thaI  sau3AI                        xmaI/pspAI
                                    hgaI                  bslI fnuDII/mvnI                         smaI
                                    ahaII/bsaHI         gsuI/bpmI   bstYI/xhoII                    scrFI
                                scrFI                  mnlI   hinPI alwI[dam-]                     nciI
                                nciI                sau3AI    hhaI/cfoI        gsuI/bpmI           dsaV
                                mspI     mspI       mboI/ndeII[dam-] mboI/ndeII[dam-]              cauII
                                hpaII    hpaII      dpnI[dam+] bstUI dpnI[dam+]                    bsaJI
                     tfiI       dsaV    naeI        dpnII[dam-] aciI dpnII[dam-]                  bsaJI
                     hinfI      cauII   cfr10I fokI alwI[dam-] bsh1236I    nlaIII      mboII  bslI avaI
3801 AAAGGTTGGG CTTCGGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT GGAGTTCTTC GCCCACCCCG GGAGATGGGG
     TTTCCAACCC GAAGCCTTAG CAAAAGGCCC TGCGGCCGAC CTACTAGGAG GTCGCGCCCC TAGAGTACGA CCTCAAGAAG CGGGTGGGGC CCTCTACCCC
                                                                           HSV1 tk terminator SmaI-PvuII  ^

                                                     hinPI
                                                     hhaI/cfoI
                                                    thaI
                                                    fnuDII/mvnI
                                       mspI         bstUI
                                       hpaII        bsh1236I
     mnlI                    bsmAI    bsaWI  nlaIV aciI
3901 GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGGA ACCCGCGCTA TGACGGCAAT AAAAAGACAG AATAAAACGC ACGGGTGTTG GGTCGTTTGT
     CTCCGATTGA CTTTGTGCCT TCCTCTGTTA TGGCCTTCCT TGGGCGCGAT ACTGCCGTTA TTTTTCTGTC TTATTTTGCG TGCCCACAAC CCAGCAAACA

                            scrFI
                            mvaI
                            ecoRII
                            dsaV
                            bstNI
                            bsaJI
                           bslI
             aciI      sau96I                                                           thaI
            thaI       nlaIV                                               haeIII/palI  fnuDII/mvnI
            fnuDII/mvnI    bsaJI                                 bslI     sau96I        bstUI
            bstUI      avaII                                 bsmAI       asuI           bsh1236I
            bsh1236I   asuI apyI[dcm+]        taqI           bsaI       nlaIV         aciI   mboII
4001 TCATAAACGC GGGGTTCGGT CCCAGGGCTG GCACTCTGTC GATACCCCAC CGAGACCCCA TTGGGGCCAA TACGCCCGCG TTTCTTCCTT TTCCCCACCC
     AGTATTTGCG CCCCAAGCCA GGGTCCCGAC CGTGAGACAG CTATGGGGTG GCTCTGGGGT AACCCCGGTT ATGCGGGCGC AAAGAAGGAA AAGGGGTGGG
```

Figure 16K

08/779457

```
                                                                                  sau96I
                                 hgiJII
                                                                                  nlaIV
                                 bsp1286
                                                                                  haeIII/palI
                                 bmyI
                                                                           sau96I
                              scrFI
                                                                                 nlaIV
                              mvaI
                                                                           hgiJII                    bslI
                              ecoRII
                                                                           eco0109I/draII        sau96I
                              dsaV
                                                                                 bsp1286                nlaIV
                              bstNI
                                                                                 bsp120I                avaII styI
                              bsaJI
                                                                                 bmyI                   asuI  ncoI
                              apyI[dcm+]
                                                                                 banII                 ppuMI  dsaI
                             bsaJI
                                                                      xcmI asuI dsaI             nlaIV bsaJI
                           sau96I    bslI
                                                                            dsaI apaI bsaJI        eco0109I/draII
           bslI            haeIII/palI fnu4HI         fnu4HI
                                                                            bsaJI asuI             tth111I/aspI nlaIII
          bslI      hphI  asuI  banII  bbvI   maeII   aciI      aciI
4101 CAACCCCCAA GTTCGGGTGA AGGCCCAGGG CTCGCAGCCA ACGTCGGGGC GGCAAGCCCG CCATAGCCAC GGGCCCCGTG GGTTAGGGAC GGGGTCCCCC
     GTTGGGGGTT CAAGCCCACT TCCGGGTCCC GAGCGTCGGT TGCAGCCCCG CCGTTCGGGC GGTATCGGTG CCCGGGGCAC CCAATCCCTG CCCCAGGGGG

                                                                                                  nlaIII
                                                                                                 styI
                                                                  sau96I                         ncoI            haeIII/palI
                                                                  avaII                          dsaI           haeI
                                                                  asuI     bsrI   ddeI           bsaJI       fokI
4201 ATGGGGAATG GTTTATGGTT CGTGGGGGTT ATTCTTTTGG GCGTTGCGTG GGGTCAGGTC CACGACTGGA CTGAGCAGAC AGACCCATGG TTTTTGGATG
     TACCCCTTAC CAAATACCAA GCACCCCCAA TAAGAAAACC CGCAACGCAC CCCAGTCCAG GTGCTGACCT GACTCGTCTG TCTGGGTACC AAAAACCTAC

                                                                                                    aciI
                                                                                                   thaI
                                                                                                   fnuDII/mvnI  hinPI
                                                                                                   bstUI        hhaI/cfoI
                                                                                             bsh1236I    nlaIV
                                             hinlI/acyI
      scrFI                                                                                       hinPI        narI
                                                   ahaII/bsaHI
      mvaI        sau96I                                                                  thaI           kasI
                            thaI         scrFI
      ecoRII                                                                              fnuDII/mvnI    hinlI/acyI
                    rsaI    fnuDII/mvnI  nciI
      dsaV                                                                                       bstUI         hgiCI
                                               mspI
      bstNI       avaII nlaIII bstUI                                                             bsh1236I      haeII
                                               hpaII
      bslI        asuI nspI   hinPI                                                             aciI           banI
      bsaJI nlaIII    nspHI  bsrI bsh1236I     dsaV hgaI     fnu4HI                          bslI hhaI/cfoI    ahaII/bsaHI
      apyI[dcm+]    aciI  csp6I  hhaI/cfoI     cauII         bbvI
4301 GCCTGGGCAT GGACCGCATG TACTGGCGCG ACACGAACAC CGGGCGTCTG TGGCTGCCAA ACACCCCCGA CCCCCAAAAA CCACCGCGCG GATTTCTGGC
     CGGACCCGTA CCTGGCGTAC ATGACCGCGC TGTGCTTGTG GCCCGCAGAC ACCGACGGTT TGTGGGGGCT GGGGGTTTTT GGTGGCGCGC CTAAAGACCG

                                                                                                                  aciI
                                                                                                                 thaI
                                                                                                                 fnuDII/mvnI
                                                                                                                 bstUI
                                                                                                                sacII/sstII
                                                                                                     haeIII/palI bsh1236I
                                                                                                    mcrI        nspBII
                                                                                                 dsaI           kspI
                                                                        hinPI                    bsaJI          dsaI
          mspI                                                                                hphI  eagI/xmaIII/eclXI
          hpaII                                                   mnlI  hhaI/cfoI           maeIII  eaeI        bsaJI
       aciI                                                   rsaI    haeII                bstEII   cfrI        aciI
      fnu4HI            bslI       sfaNI           mboII      csp6I   eco47III
4401 GCCGCCGGAC GAACTAAACC TGACTACGGC ATCTCTGCCC CTTCTTCGCT GGTACGAGGA GCGCTTTTGT TTTGTATTGG TCACCACGGC CGAGTTTCCG
     CGGCGGCCTG CTTGATTTGG ACTGATGCCG TAGAGACGGG GAAGAAGCGA CCATGCTCCT CGCGAAAACA AAACATAACC AGTGGTGCCG GCTCAAAGGC
```

Figure 16L

08/779457

```
        scrFI      nlaIV
        nciI        hgiCI
        dsaV     scrFI
        cauII mvaI
        bslI    ecoRII
       bslI     dsaV
       bsaJI    bstNI
     sau96I     bsaJI
     nlaIV  haeIII/palI
     avaII eaeI
     asuI   cfrI   bsp1286
    ppuMI mspI apyI(dcm+)                                                                                hinPI  mspI
    nlaIV hpaII    bmyI                                                                                   hhaI/cfoI
    eco0109I/draII banI                                                                            thaI     hpaII
                                                                                                          fnuDII/mvnI
                                                      mboII                                               bstUI  bsaWI
                                                      bpuAI                 fnu4HI                        bsh1236I
                                            nlaIII    bbsI                  aciI    bcgI     nlaIII  aciI    bslI        aluI
4501 CGGGACCCCG GCCAGGGCAC CTGTCCTACG AGTTGCATGA TAAAGAAGAC AGTCATAAGT GCGGCGACGA TAGTCATGCC CCGCGCCCAC CGGAAGGAGC
     GCCCTGGGGC CGGTCCCGTG GACAGGATGC TCAACGTACT ATTTCTTCTG TCAGTATTCA CGCCGCTGCT ATCAGTACGG GGCGCGGGTG GCCTTCCTCG
                  ^pBR322 sequence

                                             fnu4HI
                                            haeIII/palI                                                        aciI
                                           mcrI                                                              fnu4HI
                                           eagI/xmaIII/eclXI                  hinPI                         thaI
                                           eaeI                              thaI                           fnuDII/mvnI
                                          notI                               fnuDII/mvnI                    bstUI
                                          fnu4HI                             bstUI scfI      hinPI         hinPI
                                          aciI                               bsh1236I        hhaI/cfoI hhaI/cfoI
                                    mcrI  bsrBI aciI                       rsaI hhaI/cfoI  fnu4HI    tru9I aciI
       bsrI                     sfaNI   taqI cfrI  sfaNI                   csp6I   bslI    aciI      mseI bsh1236I
4601 TGACTGGGTT GAAGGCTCTC AAGGGCATCG GTCGAGCGGC CGCATCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT
     ACTGACCCAA CTTCCGAGAG TTCCCGTAGC CAGCTCGCCG GCGTAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA
                                                       ^delta 3
                                                        ^M13 ori

        fnu4HI
       hinPI
       hhaI/cfoI                                     hinPI
      thaI                                           hhaI/cfoI
      fnuDII/mvnI                                  rmaI                                                   mspI
      bstUI                                   hinPI  haeII                                                hpaII
      bsh1236I      aciI                      hhaI/cfoI     bsrBI                                        naeI
   maeIII bbvI  maeIII                       haeII maeI     aciI             mboII               maeII   cfr10I
4701 GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT
     CCAATGCGCG TCGCACTGGC GATGTGAACG GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA

                 nlaIV
                hgiJII
                bsp1286                                 nlaIV                                     maeII   haeIII/palI
                bmyI                                    hgiCI  taqI                              draIII  sau96I
      aluI      banII        nlaIV                      banI mnlI                      hphI      bsaAI   asuI
4801 CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC
     GTTCGAGATT TAGCCCCCGA GGGAAATCCC AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG
```

Figure 16M

```
                                        maeII pleI           tru9I     pleI                               bslI
                                       draI   hinfI maeII  mseI        hinfI          bsrI               bslI  avaI
4901 CATCGCCCTG ATAGACGGTT TTTCGCCCTT TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC
     GTAGCGGGAC TATCTGCCAA AAAGCGGGAA ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG

                                                                                              thaI
                                                                                              fnuDII/mvnI
                                                          tru9I                          tru9I   apoI tru9I
                                                          mseI               tru9I       mseI bstUI   mseI
                                               haeIII/palI           aluI    mseI    apoI     bsh1236I        sspI
5001 GGCTATTCT TTTGATTTAT AAGGATTTT GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA
     CCCGATAAGA AAACTAAATA TTCCCTAAAA CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT

        maeII                     mnlI                                                         maeII
       psp1406I                haeIII/palI                            nlaIII                      hinlI/acyI
     tru9I                     stuI                            tru9I rcaI                     ahaII/bsaHI
     mseI                      haeI                            mseI  bspHI               ddeI aatII
5101 TTAACGTTTA CAATTTTATG GTGCAGGCCT CGTGATACGC CTATTTTTAT AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT
     AATTGCAAAT GTTAAAATAC CACGTCCGGA GCACTATGCG GATAAAAATA TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA
                                ^delta 2a

                     nlaIV
                   aciI
                  thaI
                  fnuDII/mvnI
                  bstUI                                                    rcaI
                  bsh1236I                                                 bspHI
                 hinPI                                                 bsrBI   bsmAI
                 hhaI/cfoI                                             aciI nlaIII                             sspI
5201 CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT
     GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA

                mboII                                                fnu4HI
                earI/ksp632I                                         aciI                         hphI
5301 ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT
     TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA

                                        hgiAI/aspHI
                                        bsp1286                     sau3AI
                             sau3AI     bsiHKAI                     mboI/ndeII(dam-)   sau3AI
                             mboI/ndeII(dam-)                       dpnI(dam+)         mboI/ndeII(dam-)
                             dpnI(dam+) bmyI                        dpnII(dam-)        dpnI(dam+)
                             dpnII(dam-)                           bstYI/xhoII         dpnII(dam-)
                       eco57I           apaLI/snoI              bsrI         nspBII    alwI(dam-)
     hphI          sfaNI mboII(dam-)    alw44I/snoI  maeIII taqI   alwI(dam-)  aciI    bstYI/xhoII             mboII
5401 GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA
     CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT
```

08/779457

Figure 16N

08/779457

```
                                                              scrFI
                                                              nciI
                                        aciI                  mspI
                                        thaI                  hpaII
                                        fnuDII/mvnI           dsaV
                                        bstUI                 cauII
        maeII           hgiAI/aspHI     bsh1236I              hinlI/acyI
       psp1406I         bsp1286  tru9I  hinPI                 hgaI                               aciI
      xmnI              bsiHKAI  mseI   hhaI/cfoI             ahaII/bsaHI    bcgI     mcrI fnu4HI
      asp700            bmyI   ahaIII/draI
5501 GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT
     CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA

                        rsaI                                                                 fnu4HI
                        csp6I  bsrI                                                          bbvI        nlaIII
        ddeI            scaI hphI   maeIII     sfaNI      fokI   nlaIII
5601 ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG
     TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC

                                         sau96I                                              nlaIII
                                         avaII                                          sau3AI maeIII
                               sau3AI    asuI                                           mboI/ndeII(dam-)   sau3AI
            haeIII/palI        mboI/ndeII(dam-)                                         dpnI(dam+)         mboI/ndeII(dam-)
           eaeI                dpnI(dam+)                                               alwI(dam-)         dpnI(dam+)
           cfrI                dpnII(dam-)                                          nlaIII dpnII(dam-)     dpnII(dam-)
           fnu4HI              pvuI/bspCI
           aciI                mcrI  mnlI                 aluI   aciI
5701 TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT
     ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA

                                                                                        hinPI
       mspI                                                                             mstI
        hpaII                                                                          aviII/fspI    bsrI
       bsaWI                                                           fnu4HI       maeII hhaI/cfoI  tru9I
     nlaIV   aluI                                 maeIII      sfaNI    bbvI        psp1406I          mseI
5801 CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG
     GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC

                  mspI                                                                       bglI
                  hpaII                                                                      sau96I
                 scrFI                                                                       haeIII/palI
            aluI nciI        tru9I        fokI                        sau96I         hinPI asuI     mspI
           rmaI  dsaV        mseI     bsrI          aciI              avaII          hhaI/cfoI      hpaII
           maeI  cauII       aseI/asnI/vspI     mnlI                  asuI
5901 AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT
     TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA

                                          aciI
                   mspI                   thaI
                   hpaII                  fnuDII/mvnI          sau96I
                  cfr10I                  bstUI                asuI
               nlaIV  hphI            bsmAI          fnu4HI    nlaIV
              gsuI/bpmI               bsaI bsh1236I  bbvI   bsrI haeIII/palI      mnlI                      eam1105I
6001 TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG
     ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC
```

Figure 16O

```
                                                ddeI
                                             sau3AI         nlaIV
                                             mboI/ndeII[dam-]    mnlI
                                             dpnI[dam+]     hgiCI          tru9I
     pleI                                    dpnII[dam-]    banI           mseI          maeIII
     hinfI             fokI
6101 ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT
     TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA

                                                     hphI
                                                  rmaI       sau3AI
                                                sau3AI       mboI/ndeII[dam-]
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]   dpnI[dam+]
                                           tru9I dpnII[dam-] dpnII[dam-]
                                        ahaIII/draI maeI     alwI[dam-]           nlaIII                 maeII
                tru9I                tru9I      bstYI/xhoII  bstYI/xhoII          rcaI              tru9I
                mseI                 mseI mseI                                    bspHI             mseI
               ahaIII/draI                      alwI[dam-]  mboII[dam-]
6201 ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT
     TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA

                                               sau3AI
                                               mboI/ndeII[dam-]
                                               dpnI[dam+]   sau3AI             thaI
                                               dpnII[dam-]  mboI/ndeII[dam-]
                                              bstYI/xhoII   dpnI[dam+]         fnuDII/mvnI
                                        sau3AI alwI[dam-]   dpnII[dam-]        bstUI
                                        mboI/ndeII[dam-]    alwI[dam-]         bsh1236I
                                        dpnI[dam+] mboII[dam-]                hinPI       fnu4HI
               ddeI hgaI                dpnII[dam-]         bstYI/xhoII       hhaI/cfoI   bbvI
6301 TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA
     AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT

                                  sau3AI
                                  mboI/ndeII[dam-]
                                  dpnI[dam+]
                                  dpnII[dam-]
                                 alwI[dam-]
               aciI              mspI                                   bsrI                 hinPI                   rmaI
     aciI   nspBII               hpaII        aluI                   maeIII    eco57I        hhaI/cfoI               maeI
6401 CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC
     GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG

                                                                                                      fnu4HI
                                                                                          alwNI      bbvI
                                                                                         bsrI     fnu4HI
                  haeIII/palI                                                         maeIII      bbvI       bsrI
          bslI     haeI                      scfI       aciI        mnlI
6501 TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA
     ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT
```

08/779457

Figure 16P

```
                scrFI                                           mcrI                                    hgiAI/aspHI
                ncII                                            nspBII                                  bsp1286
                mspI                           mspI            fnu4HI                                   bsiHKAI
                hpaII                          hpaII           bbvI                                     bmyI
                dsaV       pleI              bsaWI            hinPI aciI                               apaLI/snoI
                cauII      hinfI           maeIII             hhaI/cfoI                                alw44I/snoI  aluI
6601 TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG
     ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC

                                                                                                   mspI
                                                        hinPI                                      hpaII
                                                        hhaI/cfoI                                  bslI     fnu4HI
                     ddeI       scfI                   haeII                           aciI       bsaWI    aciI
6701 CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC.ATTGAGAAAG CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA
     GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT

                                 scrFI
                                 mvaI         scrFI
                                 ecoRII  mvaI
                                 dsaV    ecoRII
                                 bstNI         dsaV
               hinPI  mnlI       bsaJI         bstNI                                                    taqI
               hhaI/cfoI  aluI   apyI[dcm+]    apyI[dcm+]                              mnlI  drdI    hgaI
6801 GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT
     CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA

                                                aluI                                                    tru9I
                           nlaIV                pvuII                                       hinPI      mseI
           sfaNI          aciI                  nspBII                bsrI       aciI       hhaI/cfoI  aseI/asnI/vspI
6901 TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCTGGC ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT
     AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGACCG TGCTGTCCAA AGGGCTGACC TTTCGCCCGT CACTCGCGTT GCGTTAATTA
                                                 ^delta1.PVU

                            scrFI
                            mvaI
                            ecoRII
                            dsaV
                       nlaIV bstNI
          mnlI         hgiCI apyI[dcm+]                   mspI                                aciI
       maeIII          banI bsaJI                         hpaII                              bsrBI
7001 GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA
     CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT AACACTCGCC TATTGTTAAA GTGTGTCCTT

                          tru9I
                          mseI
                         aseI/asnI/vspI
                       xmnI
       aluI     nlaIII asp700
7101 ACAGCTATGA CCATGATTAC GAATTAA
     TGTCGATACT GGTACTAATG CTTAATT

>length: 7127
```

Figure 16Q

08/779457

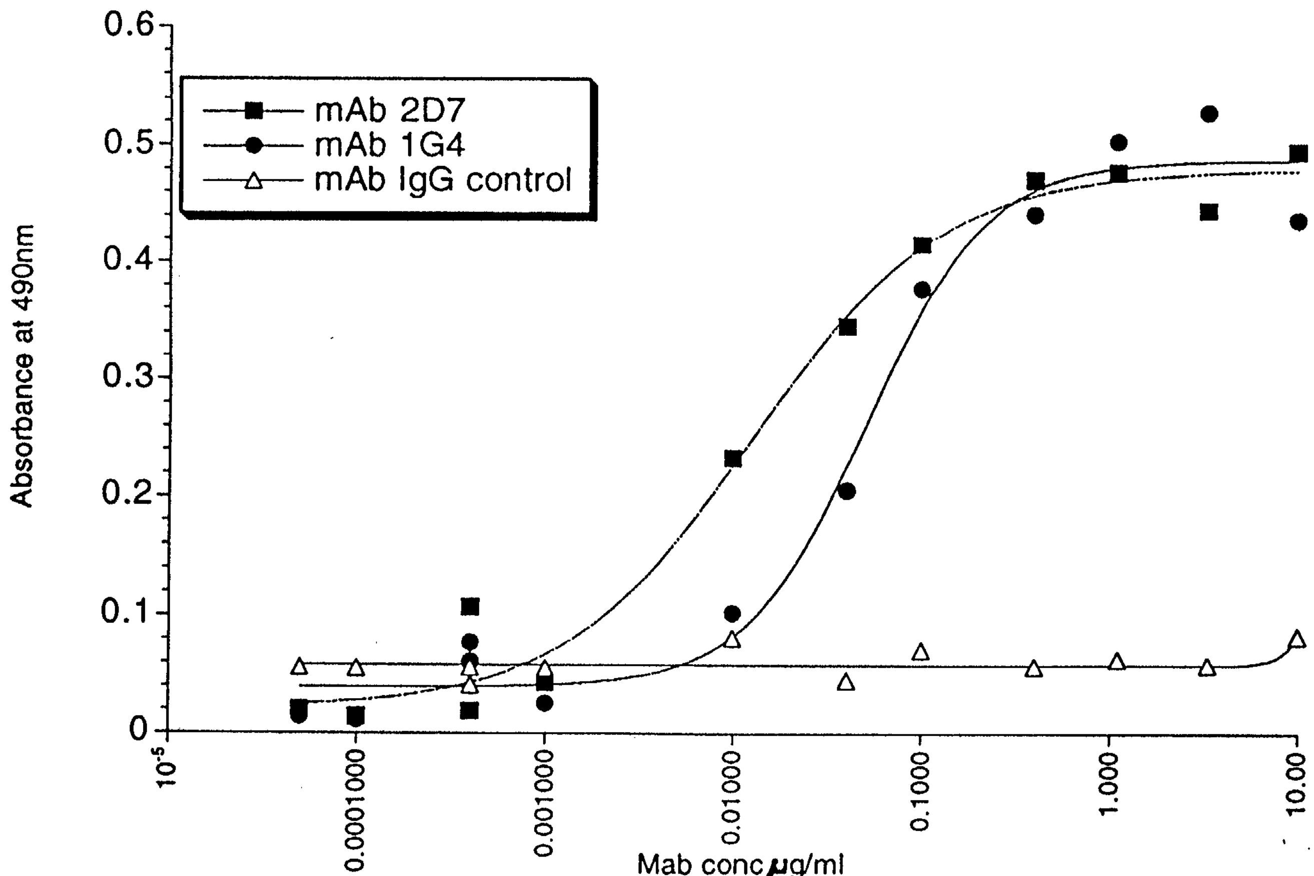

0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance at 490nm
mAb 2D7
mAb 1G4
mAb IgG control
10^{-5}
0.0001000
0.001000
0.01000
0.1000
1.000
10.00
Mab conc µg/ml

Figure 17

08/779457

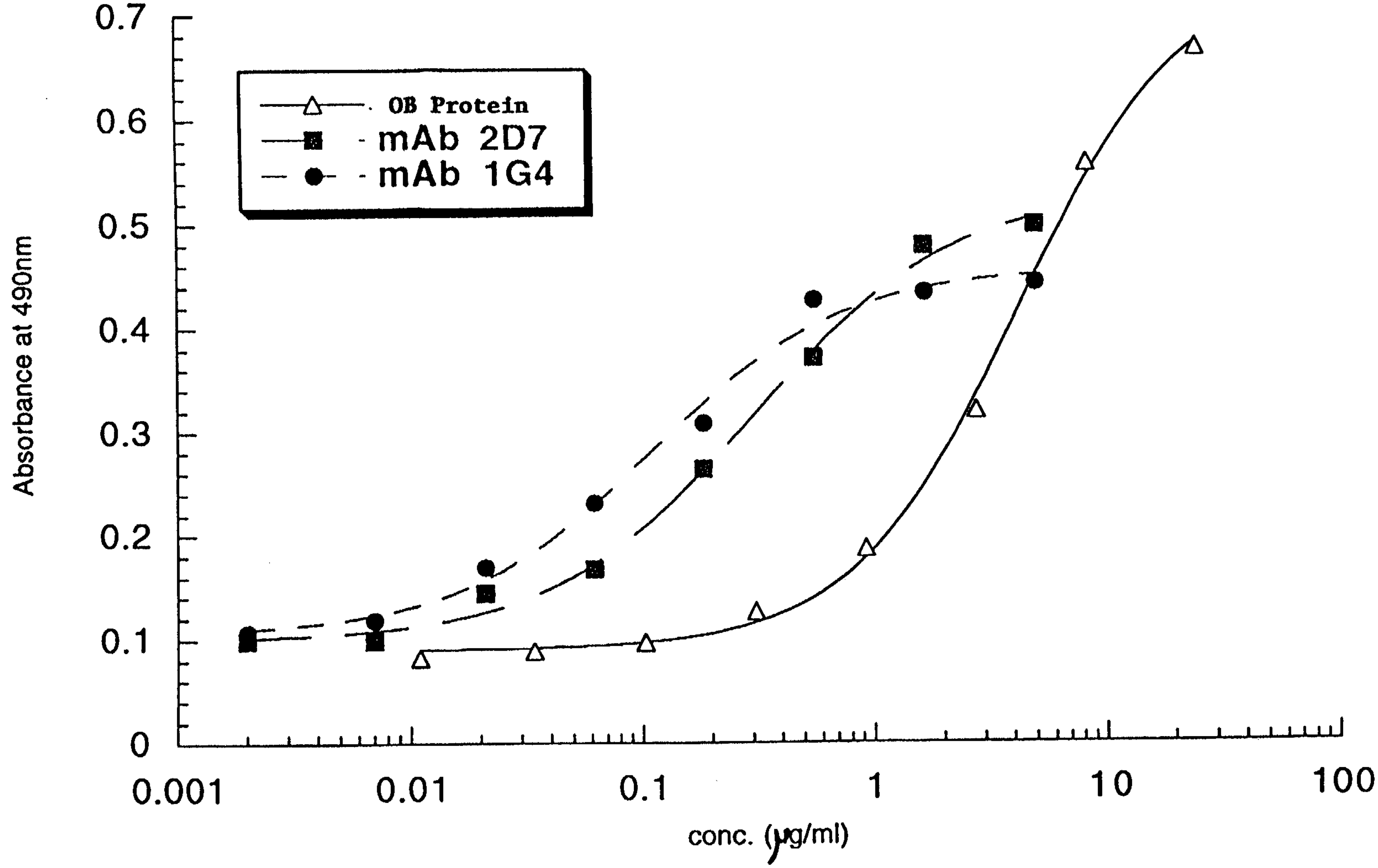


Figure 18

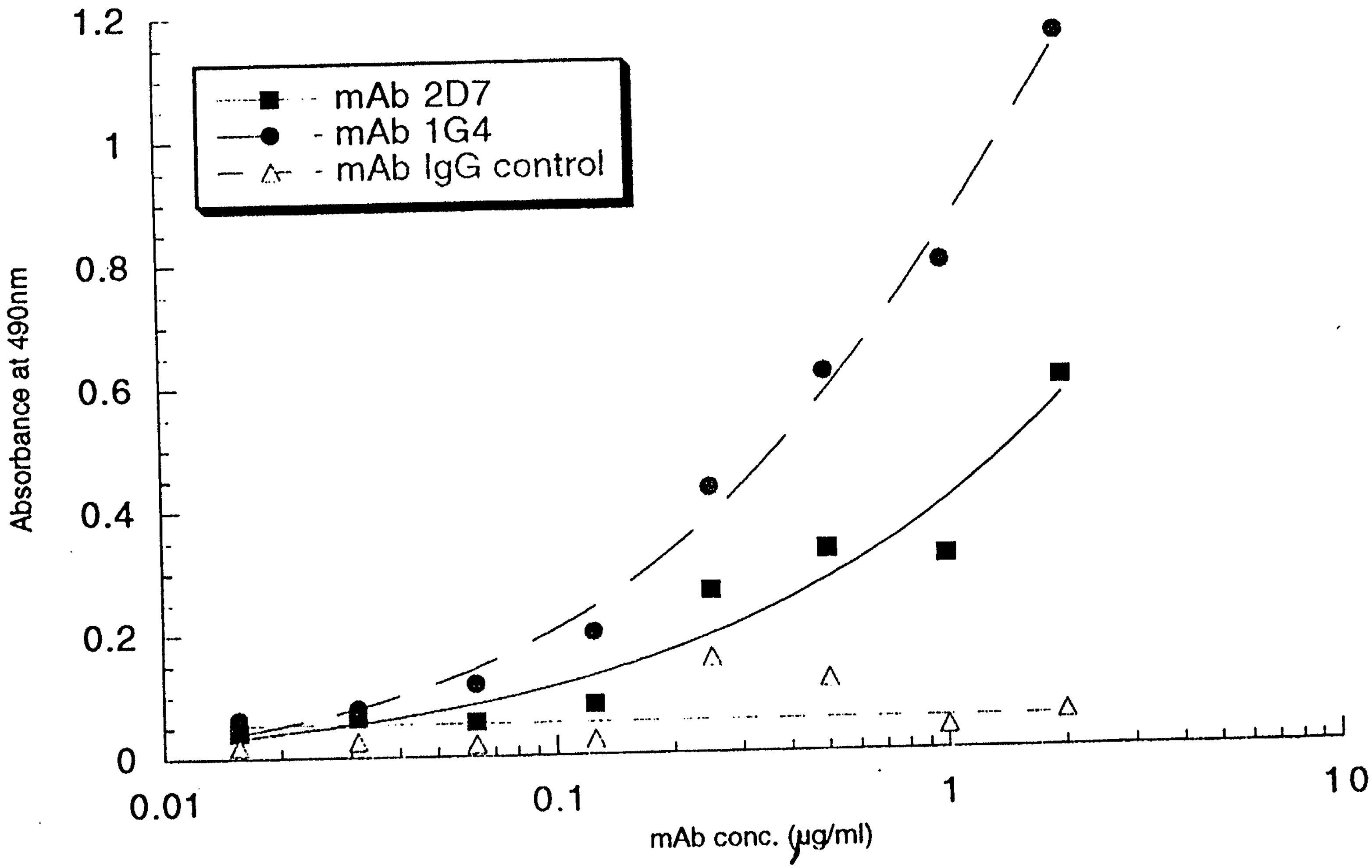


Figure 19

08/779457

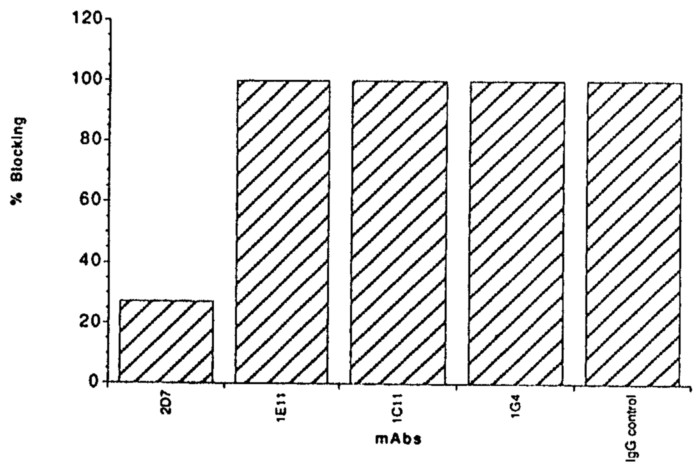


Figure 20A

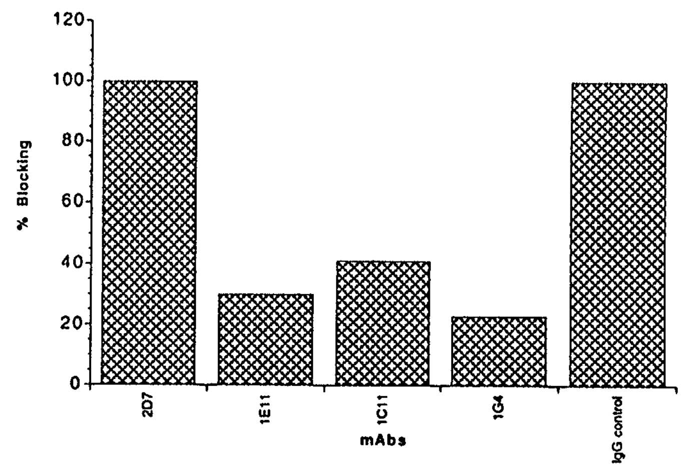


Figure 20B

08/779457

```
hWSXR   1  MICQKFCVVLLHWEFIYVITAFNLSYPITPWRFKLSCMPPNSTYDYFLLP
mWSXR   1  MMCQKFYVVLLHWEFLYVIAALNLAYPISPWKFKLFCGPPNTTDDSFLSP

       51  AGLSKNTSNSNGHYETAVEPKFNSSGTHFSNLSKTTFHCCFRSEQDRNCS
       51  AGAPNNASALKGASEAIVEAKFNSSGIYVPELSKTVFHCCFGNEQGQNCS

      101  LCADNIEGKTFVSTVNSLVFQQIDANWNIQCWLKGDLKLFICYVESLFKN
      101  ALTDNTEGKTLASVVKASVFRQLGVNWDIECWMKGDLTLFICHMEPLPKN

      151  LFRNYNYKVHLLYVLPEVLEDSPLVPQKGSFQMVHCNCSVHECCECLVPV
      151  PFKNYDSKVHLLYDLPEVIDDSPLPPLKDSFQTVQCNCSLRG-CECHVPV

      201  PTAKLNDTLLMCLKITSGGVIFQSPLMSVQPINMVKPDPPLGLHMEITDD
      200  PRAKLNYALLMYLEITSAGVSFQSPLMSLQPMLVVKPDPPLGLHMEVTDD

      251  GNLKISWSSPPLVPFPLQYQVKYSENSTTVIREADKIVSATSLLVDSILP
      250  GNLKISWDSQTMAPFPLQYQVKYLENS-TIVREAAEIVSATSLLVDSVLP

      301  GSSYEVQVRGKRLDGPGIWSDWSTPRVFTTQDVIYFPPKILTSVGSNVSF
      299  GSSYEVQVRSKRLDGSGVWSDWSSPQVFTTQDVVYFPPKILTSVGSNASF

      351  HCIYKKENKIVPSKEIVWWMNLAEKIPQSQYDVVSDHVSKVTFFNLNETK
      349  HCIYKNENQIISSKQIVWWRNLAEKIPEIQYSIVSDRVSKVTFSNLKATR

      401  PRGKFTYDAVYCCNEHECHHRYAELYVIDVNINISCETDGYLTKMTCRWS
      399  PRGKFTYDAVYCCNEQACHHRYAELYVIDVNINISCETDGYLTKMTCRWS

      451  TSTIQSLAESTLQLRYHRSSLYCSDIPSIHPISEPKDCYLQSDGFYECIF
      449  PSTIQSLVGSTVQLRYHRRSLYCPDSPSIHPTSEPKNCVLQRDGFYECVF

      501  QPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPPSSVKAEITIN
      499  QPIFLLSGYTMWIRINHSLGSLDSPPTCVLPDSVVKPLPPSNVKAEITVN

      551  IGLLKISWEKPVFPENNLQFQIRYGLSGKEVQWKMYEVYDAKSKSVSLPV
      549  TGLLKVSWEKPVFPENNLQFQIRYGLSGKEIQWKTHEVFDAKSKSASLLV

      601  PDLCAVYAVQVRCKRLDGLGYWSNWSNPAYTVVMDIKVPMRGPEFWRIIN
      599  SDLCAVYVVQVRCRRLDGLGYWSNWSSPAYTLVMDVKVPMRGPEFWRKMD

      651  GDTMKKEKNVTLLWKPLMKNDSLCSVQRYVINHHTSCNGTWSEDVGNHTK
      649  GDVTKKERNVTLLWKPLTKNDSLCSVRRYVVKHRTAHNGTWSEDVGNRTN

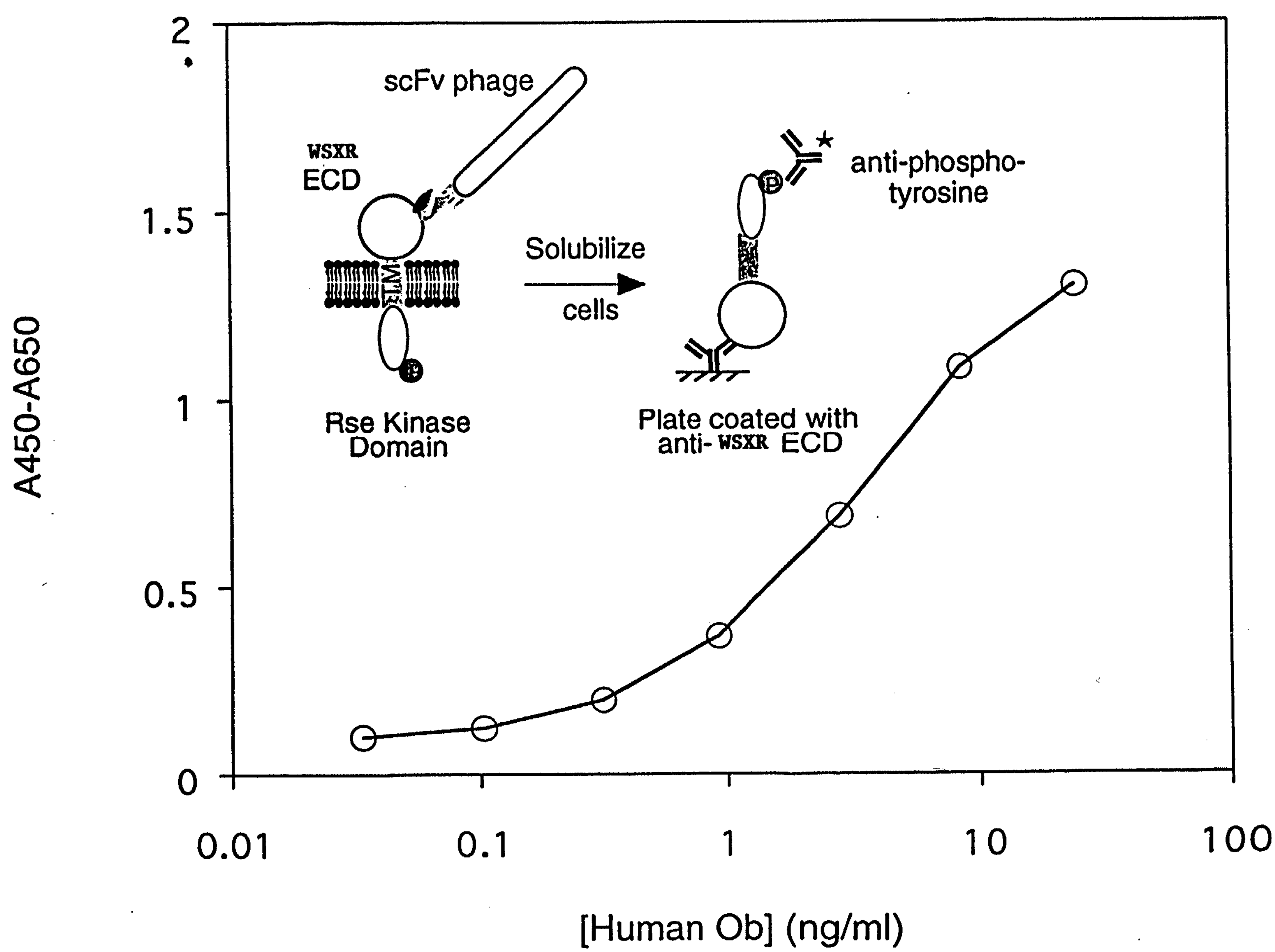
      701  FTFLWTEQAHTVTVLAINSIGASVANFNLTFSWPMSKVNIVQSLSAYPLN
      699  LTFLWTEPAHTVTVLAVNSLGASLVNFNLTFSWPMSKVSAVESLSAYPLS

      751  SSCVIVSWILSPSDYKLMYFIIEWKNLNEDGEIKWLRISSSVKKYYIHDH
      749  SSCVILSWTLSPDDYSLLYLVIEWKILNEDDGMKWLRIPSNVKKFYIHDN

      801  FIPIEKYQFSLYPIFMEGVGKPKIINSFTQDDIEKHQSDAGLYVIVPVII
      799  FIPIEKYQFSLYPVFMEGVGKPKIINGFTKDAIDKQQNDAGLYVIVPIII

      851  SSSILLLGTLLISHQRMKKLFWEDVPNPKNCSWAQGLNFQKRTDIL
      849  SSCVLLLGTLLISHQRMKKLFWDDVPNPKNCSWAQGLNFQKRTDTL
```

Figure 21



scFv phage
WSXR
ECD
Solubilize
cells
anti-phospho-
tyrosine
TM
Rse Kinase
Domain
Plate coated with
anti- WSXR ECD
A450-A650
2
1.5
1
0.5
0
0.01
0.1
1
10
100
[Human Ob] (ng/ml)

Figure 22

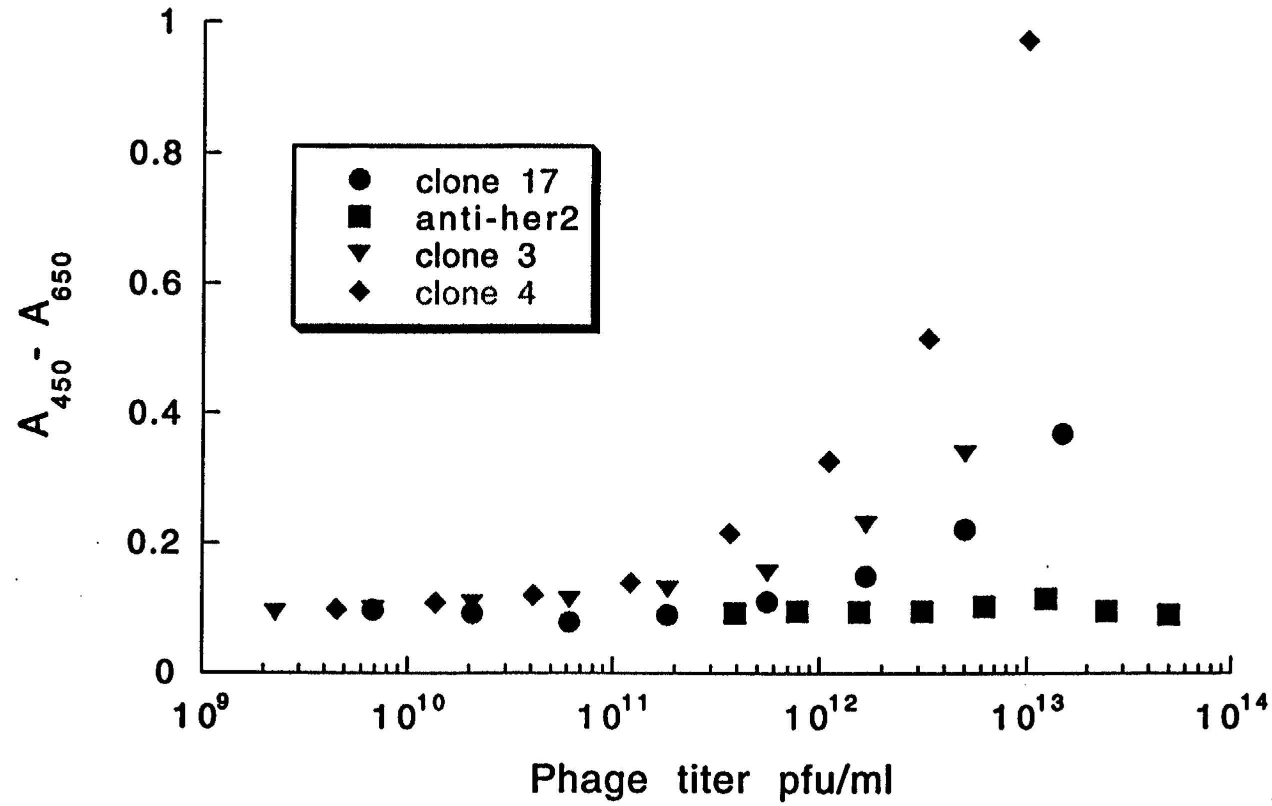
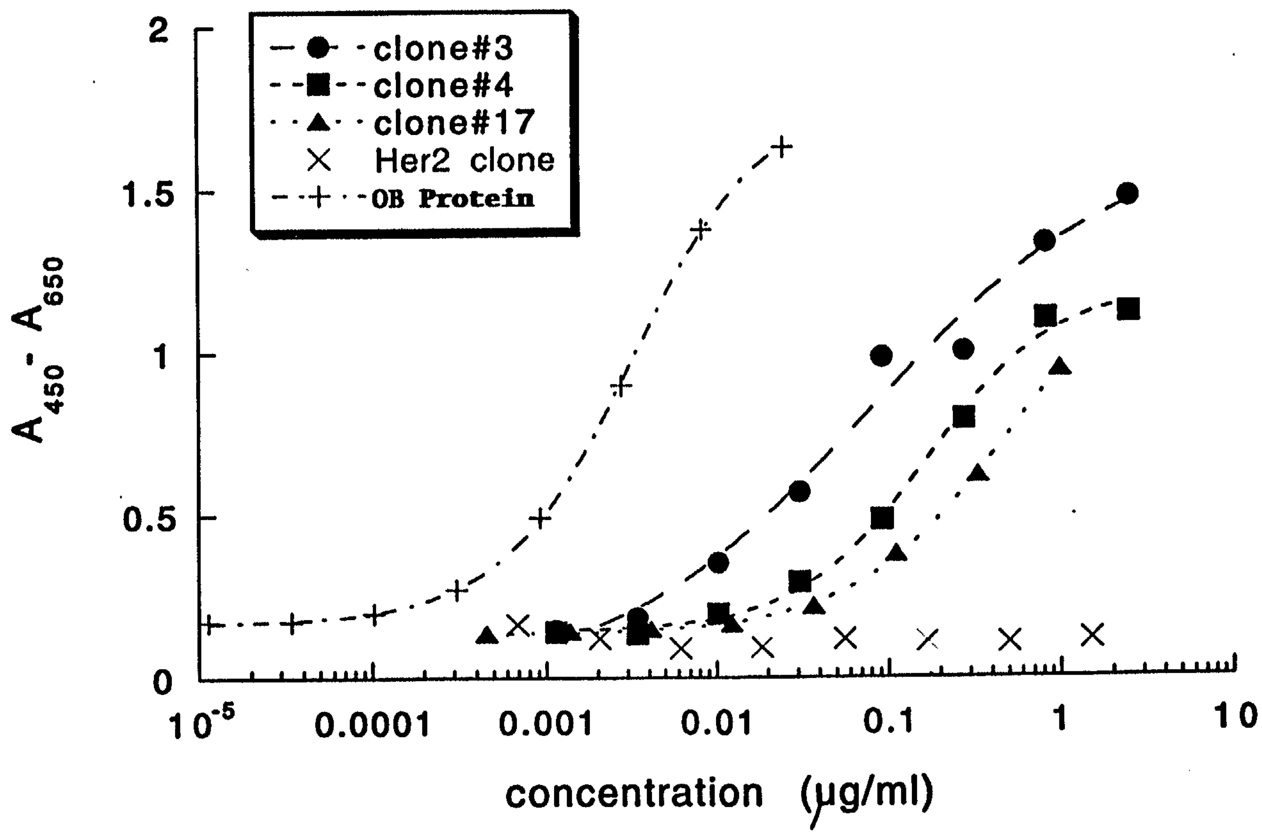


Figure 23

08/779457



clone#3
clone#4
clone#17
Her2 clone
OB Protein
2
1.5
1
0.5
0
A_{450} - A_{650}
10^{-5}
0.0001
0.001
0.01
0.1
1
10
concentration (µg/ml)

Figure 24

08/779457

```
17.scfv    1  QVRLQQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSG
3.scfv     1  EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMYWVRQAPGQGLEWMGW
4.scfv     1  EVQLVQSGAEVKKPGESLKISCQGSGFTFSSYKMNWVRQAPGKGLEWMGG
                                            CDR H1

17.scfv   51  MTWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREP
3.scfv    51  INPNSGGTNYAQKFQGRVTMTRDTSIGTAYMELSRLSSDDTAVYYCARDR
4.scfv    51  IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDR
                   CDR H2

17.scfv  101  HNTDA--------FDIWGRGTLVTVSSGGGGPGGGGSGGGGSDVVMTQSP
3.scfv   101  YYGSSAYHRGSYYMDVWGRGTLVTVSSGGGGTGGGGSGGGGS-SELTQDP
4.scfv   101  VVVPATSLRGG--MDVWGQGTTVTVSSGGGGSGGGGSGGGGSQSVLTQPA
                 CDR H3

17.scfv  143  SFLSAFVGDTITITCRASQ---GIYNYLAWYQQKPGKAPKLLIYAASTLQ
3.scfv   150  A-VSVALGQTVRITCQGDS--LRSY-YASWYQQKPGQAPVLVIYGKNNRP
4.scfv   149  S-VSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEGSKRP
                               CDR L1                   CDR L2

17.scfv  190  SGVPSRFSGSGSGTEFTLTISSLQPEDFGTYYCQQLI--SYPLTFGGGTK
3.scfv   196  SGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTK
4.scfv   198  SGVSNRFSGSKSGSTASLTISGLQAEDEADYYCSSYTTRSTR-VFGGGTK
                                                   CDR L3

17.scfv  238  VEIK
3.scfv   246  LTVL
4.scfv   247  LTVL
```

Figure 25